RESEARCH ARTICLE

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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF BECLOMETHASONE DIPROPIONATE AND KETOCONAZOLE IN COMBINED DOSAGE FORM

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Abstract:

A simple, selective and rapid reversed phase high performance liquid chromatographic (RPHPLC) method for the analysis of beclomethasone dipropionate and ketoconazole in combined dosage form has been developed and validated. The chromatographic system consisted of a quaternary pump with auto-sampler and PDA detector on a reversed-phase C18 column (250 mm × 4.6 mm i.d, 5 µm particle size) at ambient temperature with a mobile phase consisting of acetonitrile : acidic water(pH is adjusted 6.0 with acetic acid) (57 : 43, v/v) at flow rate of 0.8 ml/min and detection wavelength of 254 nm and the retention time was about 2.81 minutes for ketoconazole(KTZ) and 7.69 minutes for beclomethasone dipropionate(BCD). The method is selective and able to resolve drug peaks. The peaks of beclomethasone dipropionate and ketoconazole were well separated. The linearity of the calibration curves for each analyte in the desired concentration range was good (R^2>0.99). The method was accurate with recoveries in the range of 98-102% for both drugs and precise (%RSD of intra day variation were 0.60 – 0.78 for beclomethasone dipropionate and 0.74 - 1.05 for ketoconazole and %RSD of inter day variation were 0.89 - 1.72 for beclomethasone dipropionate and 0.76 – 1.69 for ketoconazole). The proposed method was found to be highly sensitive, accurate and precise and thatswhy this method can be used as a more convenient and efficient option for the analysis of beclomethasone dipropionate and ketoconazole in combined dosage form.

Keywords: Beclomethasone dipropionate, ketoconazole, method validation, HPLC, quantitative analysis

Introduction

Beclomethasone dipropionate (BCD) chemically is, (8S,9R,10S,11S,13S,14S,16S,17R)-9-chloro-11-hydroxy-10,13,16-trimethyl-3-oxo-17-[2-(propionyloxy)acetyl]-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-17-yl propionate (Fig.1) is an anti-inflammatory Agent.[1,2] It is official in Indian Pharmacopoeia (IP)[3], British Pharmacopoeia (BP)[4], European Pharmacopoeia (EP)[5], United States Pharmacopoeia (USP)[6] and The Japanese Pharmacopoeia (JP)[7]. Literature survey reveals HPLC[8], spectrophotometric method[9] and Stability indicating HPLC method[10] for estimation of BCD in various dosage forms. Unbound corticosteroids. cross cell membranes and bind with high affinity to specific cytoplasmic receptors The result includes inhibition of leukocyte infiltration at the site of inflammation, interference in the function of mediators of inflammatory response, suppression of humoral immune responses, and reduction in edema or scar tissue. The antiinflammatory actions of corticosteroids are thought to involve phospholipase A2 inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes.

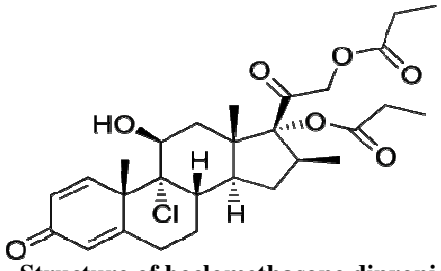

Fig 1 - Structure of beclomethasone dipropionate

Ketoconazole (KTZ) chemically is, 1-[4-(4-{[(2r,4s)-2-(2,4-dichlorophenyl)-2-(1h-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy}phenyl)piperazin-1-yl]ethan-1-one (Fig.2) is an antifungal agent[11,12]. It is official in Indian Pharmacopoeia (IP)[13], British Pharmacopoeia (BP)[14], European Pharmacopoeia (EP)[15] and United States Pharmacopoeia (USP)[16]. Literature survey reveals HPLC[17], spectrophotometric method[18], Stability indicating HPLC method[19] and colourimetric method[20] for estimation of KTZ in various dosage forms. Ketoconazole interacts with 14-α demethylase, a cytochrome p-450 enzyme necessary for the conversion of lanosterol to ergosterol. This results in inhibition of ergosterol synthesis and increased fungal cellular permeability.

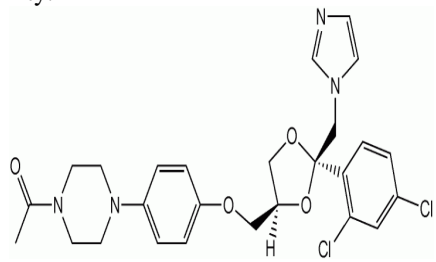

Fig 2 - Structure of ketoconazole

According to the literature survey it was found that few analytical methods such as uv and hplc methods were reported for beclomethasone dipropionate and ketoconazole individually, but not even a single method has been reported on second derivative spectroscopic method for beclomethasone dipropionate and ketoconazole in combination. So, the present work was undertaken with the aim to develop and validate an economic,simple,specific and rapid reversed-phase high performance liquid chromatographic method with high resolution according to ICH guideline for the simultaneous determination of beclomethasone dipropionate and ketoconazole in combined dosage form.

MATERIALS AND METHODS

APPARATUS

- RP-HPLC instrument equipped with a photodiode array detector (Shimadzu, LC-20AD, Japan), Phenomenex C18 (150mm x 4.6mm, i.d-5µm) column and LC-solution software were used.
- Analytical balance (Sartorius CP224S, Germany)
- Digital pH meter (LI 712 pH analyzer, Elico Ltd., Ahemedabad)
- Corning volumetric flasks (10, 50, 100 ml)
- Ultra sonic cleaner (Frontline FS 4, Mumbai, India)

REAGENTS AND MATERIALS

- Beclomethasone Dipropionate (BCD) and Ketoconazole (KTZ) were kindly supplied as a gift samples from Astrone Pharmaceuticals Ltd, Ahmedabad, Gujarat, India and Westcoast Pharmaceuticals Ltd, Ahmedabad, Gujarat, India respectively.
- HPLC grade acetonitrile, methanol and water (Finar Chemicals Ltd., Mumbai, India).
- Acetic acid (Merck Specialties Pvt. Ltd, Worli, Mumbai)
- Nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India)
- Whatman filter paper no. 41. (Whatman International Ltd., England)

PREPARATION OF SOLUTIONS & REAGENTS
PREPARATION OF ACIDIC WATER (pH 6.0) :

Acidic water was prepared by taking triple distilled HPLC grade water and its pH is adjusted to 6.0 by acitic acid.

PREPARATION OF STOCK SOLUTIONS OF BECLOMETHASONE DIPROPIONATE(BCD) AND KETOCONAZOLE(KTZ) (100 µg/ml):

An accurately weighed standard powder of beclomethasone dipropionate and ketoconazole (10 mg) were transferred to 100 ml same volumetric flask and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100 µg/ml of each.

PREPARATION OF WORKING STANDARD SOLUTIONS:

The working standard solutions of BCD and KTZ were prepared by accurately transferring (0.05, 0.1, 0.5, 1, 2, 3, 4 and 5 ml) aliquots of BCD and (0.05, 0.1, 0.5, 1, 2, 3, 4 and 5 ml) aliquots of KTZ to 10 ml volumetric flasks and were made up to mark with methanol.

PREPARATION OF MOBILE PHASE :

Mobile phase consisted of Acetonitrile: Acidic Water(pH adjusted 6.0 with Acitic acid) in the ratio of 57:43 v/v was prepared. For the preparation of 1000 mL mobile phase; 570 mL Acetonitrile, 430 mL acidic Water, were mixed thoroughly.

PREPARATION OF SAMPLE SOLUTION:

From the marketed formulation(solution) (containing beclomethasone dipropionate and ketoconazole, 0.25 mg/ml and 20 mg/ml respectively), 1 ml was transferred to 100 mL volumetric flask. The volume was adjusted up to the mark with methanol (HPLC Grade) to prepare 2.5 µg/mL of BCD and 200 µg/mL of KTZ. From the above solution, 1 mL was taken and was transferred to 10 mL volumetric flask and volume was adjusted up to the mark with methanol (HPLC Grade) to obtain solution having 0.25 µg/mL of BCD and 20 µg/mL KTZ. An aliquot (20 µl) of sample solution was injected under the operating chromatographic condition and responses were recorded.

CHROMATOGRAPHIC CONDITION :

Stationary phase:- Phenomenex C18 (150mm x 4.6mm, i.d-5µm particle size) column was used at ambient temperature.
Mobile Phase:- Acetonitrile:acidic Water (pH adjusted 6.0 with acitic acid)= (57: 43 v/v)
Flow rate:- 0.8 ml/min
Injection volume:- 20 µL
Detection: The elution was monitored at 254 nm using PDA detector.

DETERMINATION OF ANALYTICAL WAVELENGTH :

The standard solution of BCD and KTZ in combination were injected under the chromatographic condition described above. Both drugs were scanned over the range of 200 to 400 nm in spectrum mode. Detection was carried out at different wavelength and best response was achieved at 254 nm with PDA detector. This wavelength was used for detection of BCD and KTZ.

METHOD VALIDATION:

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[21].

LINEARITY:

Linearity of the method was determined by constructing calibration curves. Standard solutions of beclomethasone dipropionate and ketoconazole at different concentrations. Before injection of the solutions, the column was equilibrated for at least 30 min with the mobile phase. Accurately measured aliquots from standard stock solutions of beclomethasone dipropionate and ketoconazole (0.05, 0.1, 0.5, 1, 2, 3, 4 and 5 ml) were transferred to a series of 10 ml corning
volumetric flasks separately and the volume was made up to the mark with methanol. An aliquot (20 µl) of each solution was injected under the operating chromatographic condition as described above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentration. The calibration range was 0.5-50 µg/mL for both BCD and KTZ. The regression equations were calculated. Each response was average of three determinations.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION:

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the six replicate determinations, y intercept was calculated and the standard deviation of the y intercept was computed. From these values, the limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve

METHOD PRECISION (REPEATABILITY) :

Precision is the degree of repeatability of an analytical method under normal operational conditions. The repeatability was checked by repeatedly injecting six sample solutions of BCD (20 µg/mL) and KTZ (20 µg/mL) under the same chromatographic condition and peak area, retention time and tailing factor were measured.

INTERMEDIATE PRECISION (REPRODUCIBILITY):

The intra-day and inter-day precision (reproducibility) of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on different days over a period of 1 week for 3 different concentrations of standard solutions of BCD (10, 20 and 30 µg /mL) and KTZ (10, 20 and 30 µg /mL). The results documented as standard deviation and percent co-efficient of variation (CV, %).

SPECIFICITY:

The specificity of the method was ascertained by analyzing standard and sample solution of BCD and KTZ. The peak purity of BCD and KTZ were assessed for both the drugs.

ROBUSTNESS:

The robustness was studied by analyzing the same samples of BCD and KTZ by deliberate variations in the method parameters. The change in the responses of BCD and KTZ were noted. Robustness of the method was studied by changing the composition of mobile phase by

± 2 % of organic solvent, flow rate by ± 0.2 ml/min and

results on C18 HPLC column. In this proposed RP-

column oven temperature by 40 ± 2°C.

RESULTS AND DISCUSSION:

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for BCD and KTZ was obtained with a mobile phase comprising of acetonitrile : acidic water (pH is adjusted 6.0 with acetic acid) (57 : 43, v/v) at flow rate of 0.8 ml/min to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 254 nm based on peak area. The peak with clear baseline was obtained (Figure 3). The retention times of BCD and KTZ were 2.81 and 7.69 min, respectively. Linear correlation was obtained between area and concentration of BCD and KTZ in the concentration range of 0.5-50 µg/ml for both drugs. The low RSD value of intra-day (0.60 – 0.78 % for BCD and 0.74 - 1.05 % for KTZ) and inter-day (0.89 - 1.72 % for BCD and 0.76 – 1.69 % for KTZ) at 254 nm, reveal that proposed method is precise. The limit of detection (LOD) for BCD and KTZ were found to be 0.108 and 0.161 µg/ml and limit of quantification (LOQ) for BCD and KTZ were found to be 0.328 and 0.487 µg/ml, respectively. These data show that method is sensitive and precise for the determination of BCD and KTZ. The recovery experiment was performed by the standard addition method. The mean recoveries were 99.516 ± 0.54 and 99.823 ± 0.31 for BCD and KTZ, respectively (Table 2). The results of recovery studies indicate that the proposed method is highly accurate. The amount of BCD and KTZ present in the sample solutions were determined by fitting the responses into the regression equation of the calibration curve for BCD and KTZ and the result obtained was comparable with the corresponding labeled claim (Table 3).The proposed validated method was successfully applied to determine BCD and KTZ in combined dosage form. No interference of the excipients with the retention time of drugs appeared; hence the proposed method is applicable for the routine simultaneous estimation of BCD and KTZ.

CONCLUSION

The proposed high-performance liquid chromatographic method has been evaluated over the accuracy, precision and linearity and proved to be more convenient and effective for the quality control and identity of BCD and KTZ in pharmaceutical dosage forms.Rapid separation of BCD and KTZ was successfully attained with a relatively short retention time, provides good resolution, good peak shape, gives reliable and highly reproducible HPLC method, the linearity was observed in the concentration range of 0.5-50 µg/ml for both drugs with co-efficient of correlation, (r^2) =0.9972 and (r^2) =0.9996 for BCD and KTZ, respectively at 254 nm. The results of the analysis of combined dosage form by the proposed method are highly reproducible and reliable. The method can be used for the routine analysis of the BCD and KTZ in combined dosage form without any interference of excipients.

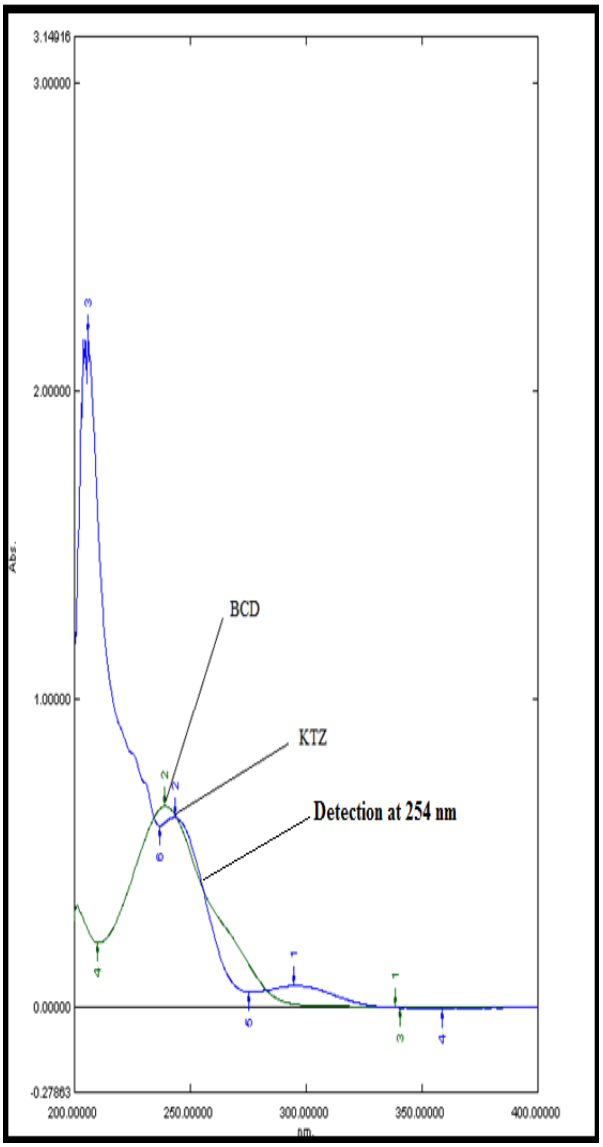

Fig 3 - Overlain UV Absorption Spectra of standard solutions of BCD (20 µg/ml) and KTZ (20 µg/ml) in methanol

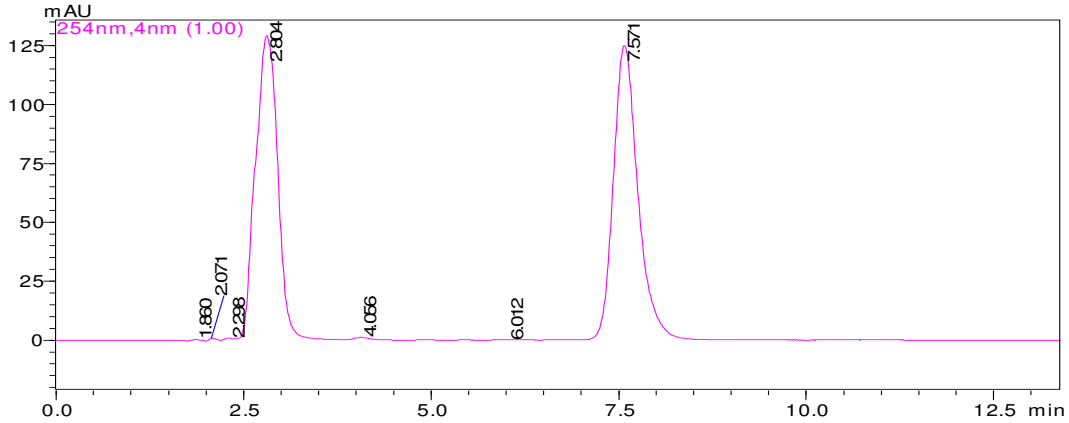

Fig 4 - Chromatogram of BCD (100µg/ml) and KTZ (100 µg/ml) at 254 nm

PARAMETERS		RP-HPLC method	
		BCD	KTZ
Detection wavelength(nm)		254	254
Beer's law limit (µg/ml)		0.5-50	0.5-50
Regression equation y = mx + c		y = 30531.60x - 716.09	y = 28118.68x + 8345.87
Slope(m)		30531.60	28118.68
Intercept(c)		716.09	8345.87
Correlation coefficient (r^2)		0.9972	0.9996
Repetability (% RSD, n = 6)		1.252	1.114
Precision (%RSD)	Intraday(%RSD)	0.60 – 0.78	0.74 - 1.05
	Interday(%RSD)	0.89 - 1.72	0.76 – 1.69
LOD(µg/ml)		0.108	0.161
LOQ(µg/ml)		0.328	0.487
Accuracy ± S. D. (% Recovery, n = 3)		99.516 ± 0.54	99.823 ± 0.31

Table 1 - Data Showing Linearity and Precision of the Developed Method

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % Recovery ± RSD
BCD	I	0.5	50 %	98.33 ± 0.81
	II	0.5	100 %	99 ± 0.39
	III	0.5	150 %	101.22 ± 0.41
KTZ	I	20	50 %	101.76 ± 0.25
	II	20	100 %	98.23 ± 0.26
	III	20	150 %	99.48 ± 0.41

Table 2 - Recovery Data of BCD and KTZ

Drug	Label Claim (mg)	Amount Found (mg)	% Label claim ± S.D. (n=6)
BCD	0.25	0.2505	100.238 ± 1.68
KTZ	20	19.849	99.247 ± 0.652

Table 3 - Results of Analysis of Tablet Dosage Forms Containing BCD and KTZ

Parameters	BCD ± RSD (n = 6)	KTZ ± RSD (n = 6)
Retention time (min)	7.69 ± 0.5069	2.81 ± 1.6692
Tailing factor	1.405 ± 0.507	1.204 ± 1.669
Theoretical plates	2421 ± 0.582	2258 ± 1.307
Resolution	4.16 ± 0.324	

Table 4 - System suitability test parameters for the proposed RP-HPLC method

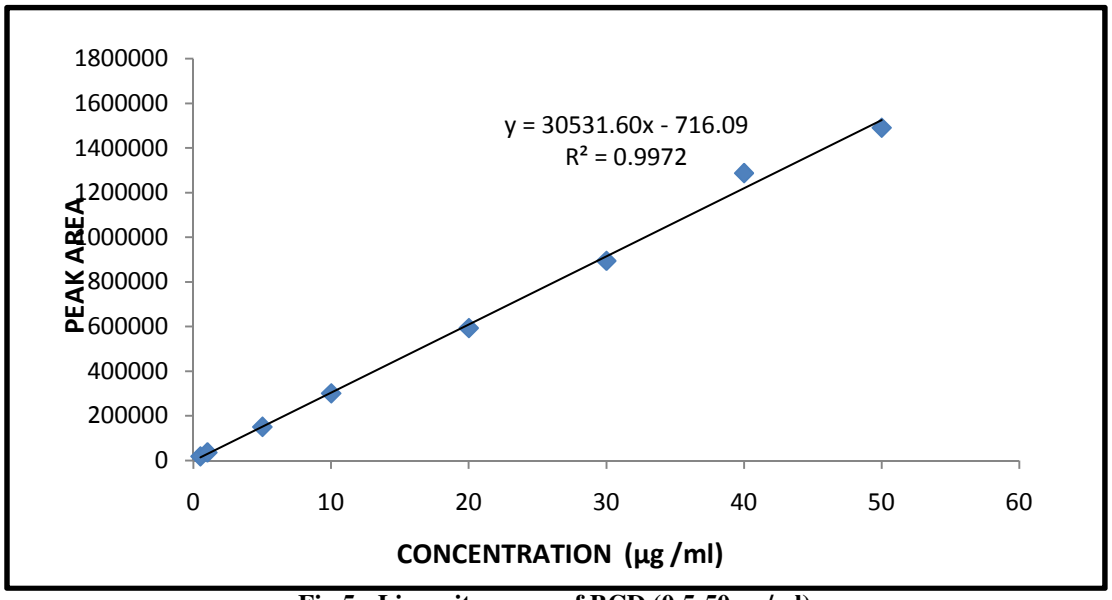

Fig 5 - Linearity curve of BCD (0.5-50 µg/ml)

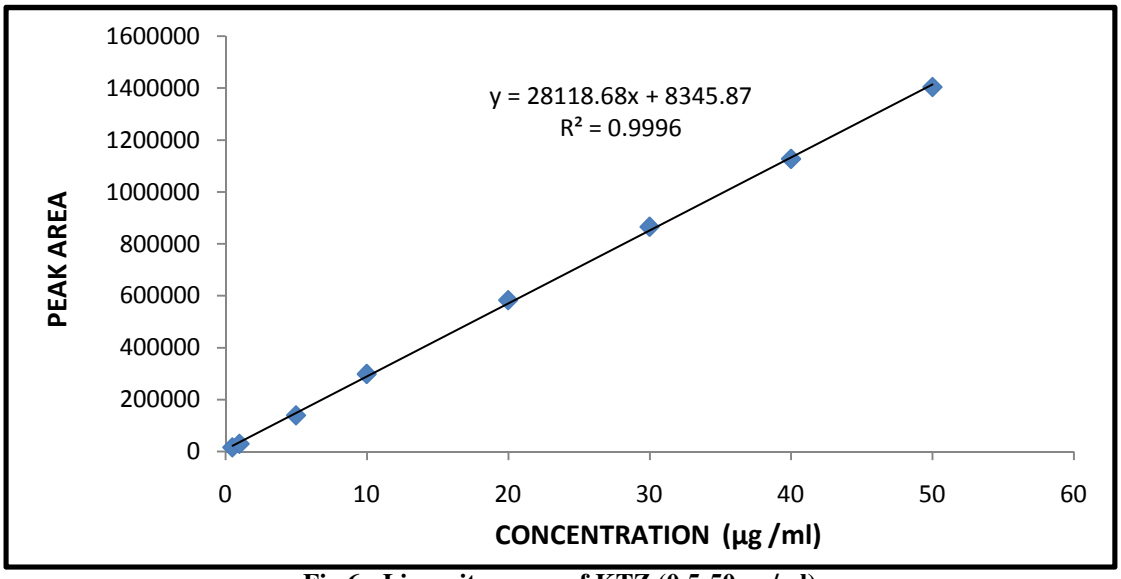

Fig 6 - Linearity curve of KTZ (0.5-50 µg/ml)

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