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IN-VITRO STUDY OF STABILITY, QUALITY AND QUANTITY OF SOME CLINICALLY AND NON-CLINICALLY USED CORTISONES FROM PHARMACEUTICAL PREPARATIONS

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ABSTRACT

The selected cortisone drugs, Betamethasone (BET), Dexamethasone (DEX), Triamcinolone (TRI) contain ketone group which have very potent synthetic glucocorticoid activity. These drugs are very much effective on a wide range of Systemic, ocular and skin diseases. In present work the effect of normal and accelerated storage conditions such as temperature and humidity was carried out to observe chemical and physical change. The stability of drug was observed by kept it under 25- 40 °C and humidity 60-75 % for 6-12 months time period. For quantitative spectrophotometric determination, the methods were developed with optimized conditions, like natural absorbance and derivitized by suitable derivatizing reagent: 2, 4 dinitrophenylhydrazine (DNPH) to cause increase in the molar absorptivity or bathochromic shift for selected drugs. In present method the solution of DNPH (0.5%) was freshly prepared in acidic alcohol mixture (2:98) and 0.01% alcoholic solution of BET, DEX and TRI were prepared. For derivatization the alcoholic solution of (0.2-1mL) containing DEX, BET and TRI 20-100 µg/mL were separately transferred to 5mL calibrated stopper volumetric flask than 1mL of DNPH (0.5%) was added to DEX and BET while 1.5 mL (0.5%) in TRI followed by addition of Acetate Buffer pH5 and pH3 to DEX and BET respectively and ammonia/ ammonium chloride buffer with pH 11 to TRI. The mixtures were heated on water bath at 95 °C for 20-30 minutes. Optimized parameters as reagent concentration, ordering mixing of reagents, Interference of associated excipients, heating time &

temperature, stability of derivative, solvent effect and pH (buffers) effects were being to consider. The absorbance of each of above derivative was measured as 480,482,487 nm against reagent blank. Which were prepared in similar way out of adding of DEX, TRI and BET respectively with molar extinction coefficient of 965.5, 1642.6 and 2461.4 $mole^{-1}$ cm^{-1}, respectively. For the effect variation in the correlation of selected drugs, according to law of Beer's, 20-100 micro gram per milliliter was the range of desired concentration that proved the beer's law and also help for the determination of selected drug i.e Dexamethasone, Bethamethasone and Trimicinolone and the value was R2 0.9994-0.9997. The law of sensitivity by sandell's given the value of DEX= 0.224, TRI=0.18 and BET=0.37µg /mL. The effect of possible additive was observed and % relative error was not more than 6.2%. The developed method was also useful in determination of stability of different formulations containing cortisones. Assay of different brands was conducted by spectrophotometer and results were calculated as BET, 97.8-100.7%, DEX 98.7-103% TRI 98.8- 99.1% .Recovery was in the range of 90-110% matching with BP specifications. From above stability analysis and evaluation with RSD% for creams 0.945, for injections 1.8 for eye drops 1.55 averages of pharmaceutical preparations which have been found stable. Therefore, it has been concluded that stability study of cortisone found equally accurate, reproducible, robust, and could be applied directly to the pharmaceutical preparations.

Keywords: Cortisone Drugs, Betamethasone (BET), Dexamethasone (DEX), Triamcinolone

INTRODUCTION

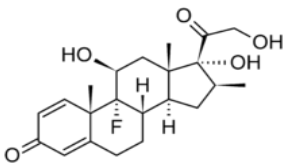
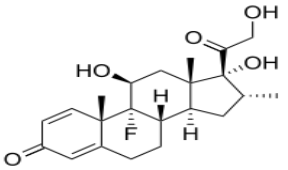
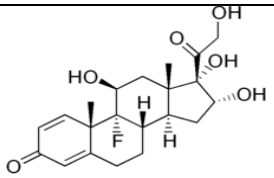
Betamethasone is chemically recognized as a N-4-tert-butylbenzyl-N-methyl-1-naphthalene methylamine hydrochloride. Physically it is white, odorless, crystalline powder generously soluble in certain volatile substances including alcohols i.e, methanol, ethanol, and chloroform. Mostly recommended in varrious skin conditions including; Inflammatory dermatoses such as atopic or seborrehic dertmatitis, neurodermatitis, anodenital pruritis, psorisi and dryness (Xerosis). Mostly its avoided to be used when there is expected hypersensitive reactions. The unwanted effects might occurs a certain severe cardiovascular complications, nervous conditions i.e, lethargy, tiredness, lack of sleep, skin disorder, abnormality in mensturation cycle, edema, eye complication, skeletal muscle spasm etc. **[1-7]**.

Dexamethasone is available as a crystalline powder with white color **[8]**. The drug is easily solublized in water and methanol.

However, it is sparingly soluble in ethanol. **[9]**. Dexamethasone has pKa values of 6.00 **[10]** and bears a m.p of 233-235°C. It is first and foremost used to manage certain inflammatory conditions and also used as immunosuppressant drug. However, the other indications includes the treatment of a variety of skin diseases, allergies, immune system disturbances anti neoplastic, anti asthmatic, cerebral edema etc **[11**].

Triamcinolone is whitish power occurs in crystalline form. Physically its molecular weight is 434.5 gram per mole. However it is soluble in water and bearing melting point of 292-294°C**. [12].** Triamcinolone is mostly employed in the treatment of Gout. However, it is also used in different conditions of bone and joints, hair loss, skin lesions, dermatological disorders. Furthermore, Adrenocortico insufficiency, dermatologic diseases, endocrine disorders, gastrointestinal disorders, blood disorders and malignant conditions.. Most commonly occurring ADRs includes; Arrhythmias, pulmonary edema, cardiovascular complications, Nervous disorders, Skin disorders, GI upset , Heamorrhage, diabetes, Edema, LFT changes etc **[13]**.

A previous study showed about that how nystatin and triamcinolone was determined in a topical dosage form of formulations. The 304nm and 240nm wavelengths were used in current study of both drugs by simultaneous equation method. The current method was also applied for determination of assay of those drugs which were available as marketed formulations and the ranges was 97-98% of the labeled value for both drugs i.e. Nystatin and Triamcinolone acetonide **[14]**. Another study was to determine the three drugs i.e. Dexamethasone (DEX), the polymyxin B(PLX) antibiotic and trimethoprim (TMP) by four different spectrophotometric methods which are as follows: partial least squares (PLS) 1 and 2, multivariate method, ratio spectrum-zero derivative methods and principal regression algorithm method. Internal validation method was applied for their calibrations **[15]**. A study was used to determine the estimation of Betamethasone in different dosage forms and in bulk with the help of spectrophotometric method. In this method bluish green chromophore with 0.03% of potassium chromate and concentrated sulphuric acid was formulated and at 601 nm of absorption was formed. The above method was also used for the quantification of betamethasone and it is validated as per ICH guidelines **[16]**.

 **Betamethasone**	 **Dexamethasone**	 **Triamcinolone**

EXPERIMENTAL METHODOLOGY

In this study, the pharmaceutical and analytical solvents, reagents and chemicals were used. Distillation plant made up of mostly glass was used for preparing of double distilled water which was used in current study. The sources of Betamethasone, dexamethasone and Triamcinolone were different from each other. Betamethasone (BET) was collected from glaxo smith kline Pharma Company located in Karachi; Dexamethasone (DEX) was collected from Pfizer Pharma Company located in Karachi, Triamcinolone (TRI) was collected from Bosch pharma company situated in Karachi, 2-4-di-nitro-phenylhydrazine (DNPH) and acetic acid were collected from E. Merck Pharma company (Germany), Ethanol and Sodium acetate was collected from Fluka Switzerland and BDH united kingdom respectively.

1 M solution of hydrochloric acid was used at pH 1 to 10 for the preparation of buffer solution. 1 M solution of potassium chloride was used at pH 1 to 10 for the preparation of buffer solution. 1 M solution of acetic acid was used at pH 1 to 10 for the preparation of buffer solution, 1 M solution of sodium acetate was used at pH 1 to 10 for the preparation of buffer solution. 1 M solution of sodium carbonate was used at pH 1 to 10 for the preparation of buffer solution. 1 M solution of ammonium chloride was used at pH 1 to 10 for the preparation of buffer solution and 1 M solution of ammonium was also used at pH 1 to 10 for the preparation of buffer solution. 0.1 to 0.5mL of aqueous solution was prepared which contained 1 µg of betamethasone, then it was transferred to volumetric flask that was also calibrated and the volume was 5mL, after that another 1 mL DNPH (0.5% in ethanol/methanol + 2 ml HCl 32% w/v) was added in aqueous solution, in the last 0.5 mL of acetate buffer (pH=3) was added. In next step, 95 degree Celsius heated was given to above solution at water bath for approximated half an hour. After heating, the temperature was dropped up to room temperature and ultimately our solutions were cooled and ethanol was used for adjustment of solution up to the required mark. At 487 nm, the absorbance of solution was measured with

the help of spectrophotometer against the reagent that was prepared by only accepting the addition of betamethasone in a same way.

Dexamethasone determination with analytical method

0.1 to 0.5mL of aqueous solution was prepared which contained 1 µg of dexamethasone, then it was transferred to volumetric flask that was also calibrated and the volume was 5 mL, after that another 1.5 mL DNPH (1% in ethanol w/v) was added in aqueous solution, in the last 0.5 mL of acetate buffer (pH=6) was added. In next step, 95 degree Celsius heated was given to above solution at water bath for approximated half an hour. After heating, the temperature was dropped up to room temperature and ultimately our solutions were cooled and ethanol was used for adjustment of solution up to the required mark. At 480 nm, the absorbance of solution was measured with the help of spectrophotometer against the reagent that was prepared by only missing the addition of dexamethasone in a same way.

Triamcinolone determination with analytical method

0.1 to 0.5mL of methanolic solution was prepared which contained 1 mL of Triamcinolone, then it was transferred to volumetric flask that was also calibrated and the volume was 5 mL, after that another 1.5 mL DNPH (1% in ethanol w/v) was added in aqueous solution, in the last 0.5 mL of sodium bicarbonate buffer (pH=11) was added. In next step, 95 degree Celsius heated was given to above solution at water bath for approximated 20 minutes. After heating, the temperature was dropped up to room temperature and ultimately our solutions were cooled and ethanol was used for adjustment of solution up to the required mark. At 482 nm, the absorbance of solution was measured with the help of spectrophotometer against the reagent that was prepared by only accepting the addition of triamcinolone in a same way.

Stability

In the present work some of the steroids such as Betamethasone (BET), dexamethasone (Dex), and Triamcinolone (TRI) will be subjected to the study. The selected steroids were examined qualitatively in their commercial brands through color, clarity, sterility, pH and filled volume testing .Factors affecting the stability: **Environmental factors (**Temperature, Oxygen (oxidation), Carbon di oxide, Light, Moisture), **Drugs or excipients in the dosage forms (**Particle size of the drug, pH of vehicle or medium, Additives **Microbial contamination, Trace metallic contamination, Leaching from containers or other during manufacturing,** The different pharmaceutical preparations

including creams, parenteral preparation, eye/ear drops containing above mentioned drugs will be collected and examined. The priority will be given to all pharmaceutical preparations present in above mentioned market. Physical examinations test of the product. Chemical assay or quality examination. Sterility tests if the product is sterile. In the present work we concern with the above 1st and second after following the criteria of British pharmacopeia 2010.

RESULTS AND DISCUSSION

Qualitative Examinations

Betamethasone Sodium Phosphate

Betamethasone sodium phosphate was available as injectables, eye drop and creams, all brands of injectables were colorless, clear and sterile. pH was within specified range according to B.P sps; i.e for Betnesol injection 8.4. Rekabson 8.1 volume was more than 1 ml. Eye / ear drops pH Probeta N 7.1 Betatek 8.0, Betnesol 7.8, Creams are available as 0.1% and the weight was within the range and color was observed white.

Dexamethasone Sodium phosphate

Dexamethasone Sodium phosphate was available as injectables, eye drop and cream. All brands of injectables were colorless, clear and sterile, pH was within specified range according to B.P sps; i.e for Decadron 7.5, D-cort 7.2, Dexamethasone iraz 7.9 and Dexadrin 8.0 volume was more than 1 ml. For eye /ear drops were with pH of Dexachlor 7.5, Dexamex 7.6, Dexoptic7.0, Ocudex 7.5 and Medidex 7.1.Creams are available as 0.1% and the weight is 15 gram. All brands were white in color and weight was also within specified range.

Triamcinolone acetonide

Triamcinolone acetonide was available as injectables and creams. All brands of injectables were colorless, clear and sterile; pH of K-kort 7.3, Lonacort 7.0. Creams were available as concentration 1 mg/g and the weight was 10 gram. All brands were white in color and weight was also within specified range.

Stability and Physical Characteristics

After following the specification of standard monograph British pharmacopeia (bp 2010).The storage temperature under certain humidity few samples were analyzed and the following were on observation. Accelerated and long term both studies at temperature 40-25˚C and humidity was 75-60 for 6 and 12 month **(Table 1-5)**.

Simple Spectrophotometric Determination of Bet, Dex, Tri in Pharmaceutical Preparations

In current study, whatever the reagents and chemicals was used it was so much elementary and low cost. (BET), (DEX),

(TRI), with 2,4 dinitrophenyl hydrazine (DNPH) to form an hydrazone derivative, which absorbs maximally 487,480,482nm and the molar absorptivity was also obtained with shift of bathochromic i.e 2461.4, 965.5, and 1642.6 $mole^{-1}$ cm^{-1}, respectively.

Analysis of different wavelength of selected drugs

In analysis of different reagents and chemicals wavelength determination with their absorbance was very much important exercise for their quantitative analysis. For derivatizing agent some parameters were important and necessary like when the derivative of analyte was absorb at some wavelength, it was also necessary that at the same or close region the derivatizing agent was not absorbed, it was absorbed than it may leads to defect in absorption because for quantitative absorption the sufficient amount of derivatizing agent was required. So to avoid the above situation, it was necessary that always select that wavelength where maximum absorbance of analyte derivative and minimum absorbance of derivatizing reagent was obtained.

At wavelength starting from 230nm up to 550nm, the absorbance of betamethasone was analyzed with 4µg mL^{-1} value of absorbance as DNPH derivative, in next step, 95 degree Celsius heat was given to above solution at water bath for approximated half an hour buffer pH 6. So finally at 487 nm wavelength the absorbance was maximum with respect to blank reagent so it was called as optimum wavelength. At wavelength starting from 250nm up to 550nm, the absorbance of dexamethasone was analyzed with 4µg mL^{-1} value of absorbance as DNPH derivative, in next step, 95 degree Celsius heat was given to above solution at water bath for approximated 20 minutes buffer pH 3. So finally at 480 nm wavelength the absorbance was maximum with respect to blank reagent so it was considered as optimum wavelength. At wavelength starting from 230nm up to 550nm, the absorbance of triaminocinolone was analysed with 2µg mL^{-1} value of absorbance as DNPH derivative, In next step, 95 degree Celsius heat was given to above solution at water bath for approximated 20 minutes buffer pH 11. So finally at 482 nm wavelength the absorbance was maximum with respect to blank reagent so it was called as optimal wavelength.

Consequence of reagent concentration

At 5 µg mL^{-1} concentration of betamethasone, dexamethasone and Triamcinolone, checked the consequence of various concentrations of DNPH. The ethanolic DNPH was also examined with 1 to 3 mL at the interval

between 0.5 to 1.5 mL and the wavelength was obtained at 487,480,482 nm respectively.

Steps of reagent mixing

In derivatizing process the order of mixing the reagents has important role in accuracy and maximum absorbance of wavelength. In current study it was studied that different quantity of selected glucocorticoids with incorporation of buffers (0.5-1mL) was added in 5mL of volumetric flask that was calibrated after that appropriate reagent i.e. 0.5-1% ethanolic DNPH w/v was also added for decreasing the absorbance. If first reagent taken, secondly buffer taken and lastly drug solution than it give the lower value of absorbance wavelength. If reagent solution was added first and then buffer solution add in the solution of drug than ultimately the absorbance was maximum and the volume was heated on water bath and adjusted with the help if water up to the required mark.

Parameters for the Formation of Stable derivatives

The most important parameters for the formation of stable derivatives are temperature and appropriate time so that to achieve the maximum absorbance wavelength. At 5 µg/ mL of betamethasone, dexamethasone and Triamcinolone solution, the effect of time was observed in the presence of 0.5% DNPH at different absorbance such as 487,480,482nm for about half an hour with an interval of five minutes. After heating the solution for 20-30 minutes maximum absorbance was obtained, so finally it was concluded that at 95 degree Celsius was the optimal degree of heating and 20-30 minutes was optimal time of heating the derivatization. (Fig No: 1)

Solvents effects

At 05µg/mL of betamethasone, dexamethasone and Triamcinolone, the effect of different solvents was observed such as propan-1-ol, methyl alcohol, butan-1-ol, amyl alcohol, iso amyl alcohol, aceto nitrile, ethyl acetate, toluene, nitrobenzene and carbon tetra chloride. About 1 to 2 mL of the above solvents was incorporated in the solution of 1 to 1.5mL 0.5% ethanol DNPH, after that buffer was added with volume of 0.5 mL and then applies optimum time and heat to the solution. Finally volume was adjusted with water or alcohol and the effect was observed with maximum absorbance.

pH Effect

At 05µg/mL of betamethasone, dexamethasone and triaminocinolone solution with DNPH with controlled condition of heating and time observed the consequence of adding the buffers of 1 M with the pH range starting from 1 up to 10 and check the absorbance of the solution that either at which

pH the absorbance was increased like at 3, 6 and 10.

Analysis of Betamethasone from pharmaceutical preparations

0.01 gram of betamethasone sample that was obtained from GSK Company was added in 100 mL of water solution. The solution contained 0.8mL of solution was transferred in 10mL of calibrated volumetric flask then it was determined by following general procedure as mentioned in 3.3.1. Each sample that contained the specific quantity of betamethasone was also determined by using the external calibration curve.

Analysis of Dexamethasone from pharmaceutical preparations

A total of 30 different samples of dexamethasone in decadron were analyzed that was collected from MSD Pharma Company situated in Karachi. 0.01 gram of dexamethasone sample that was obtained from different Pharma Company was added in 100 mL of water solution. The solution contained 0.6mL of solution was transferred in 10mL of calibrated volumetric flask then it was determined by following general procedure as mentioned in 3.3.2. Each sample that contained the specific quantity of dexamethasone was also determined by using the external calibration curve.

Analysis of TRI from pharmaceutical preparations

0.1gram of TRI sample was obtained that was equivalent and added in 20 mL of ethanolic solution. After that the solution was filtered by using whattman filter paper No 41. The filtrate was obtained and it was marked up to 100 mL by adding ethanol and the drug solution aliquots were treated as mentioned in 3.3.3. Each sample that contained the specific quantity of TRI was also determined by using the external calibration curve.

Table 1: Long test results of Dexachlor Eye Drops

DEXACHLOR EYE DROPS long test Result (25±2°C/60±5%RH)

Product Name DEXACHLORE EYE DROPS		A.I DEXAMETHASONE				
STABILITY CONDITIONS		Temperature: 25±2 Relative Humidity 60%				
Parameter	Specifications	Months				
		INITIAL	3	6	9	12
	Clear	Conform	Conform	Conform	Conform	Conform
Appearance	Colorless	Conform	Conform	Conform	Conform	Conform
pH	7–7.5	7.49	7.49	7.31	7.18	7.26
Assay	90% --110%	101.46%	101.02%	99.26%	98.46%	97.94%

Table 2: Assay results of Dexachlor eye drops

DEXACHLOR EYE DROPS long test Result (25±2°C/60±5%RH)

Time	Assay %
Initial	101.46
3M, %	101.02
6M, %	99.26
9M, %	98.46
12M, %	97.94
Mean	99.628
STD	1.552649

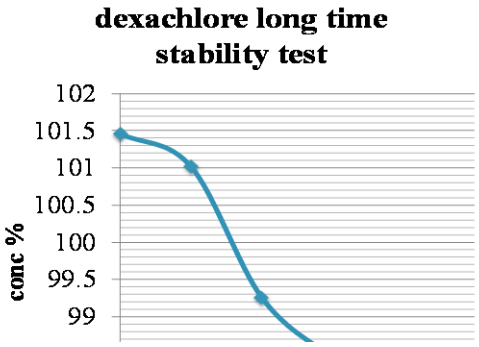


Table 3: Triamcinolone long test results

Products: K-KORT long test Result (25±2°C/60±5%RH)

STABILITY CONDITIONS		Temperature:25°C Relative Humidity 60% - 65%				
Parameter	Specifications	Months				
		INITIAL	3	6	9	12
Appearance	White to cream colored suspension	Pass	Pass	Pass	Pass	Pass
pH	5.0 – 7.5	6.20	6.20	6.19	6.17	6.18
Assay	90– 110%	98.75%	98.21%	97.46%	96.32%	94.19%

Table 4: Fusil B cream long test results

Products: Fusil B Cream long test Result (25±2°C/60±5%RH

STABILITY CONDITIONS		Temperature:25°C Relative Humidity 60% -+5%				
Parameter	Specifications	Months				
		INITIAL	3	6	9	12
Appearance	Homogenous	Conform	Conform	Conform	Conform	Conform
	White colored cream	Conform	Conform	Conform	Conform	Conform
pH	3.5–6	4.12	4.09	4.07	4.01	3.99

Table 5: Decadron Inj Accelerated Stability Test Result

Products: Decadron inj Accelerated Stability test result

STABILITY CONDITIONS		Temperature:40°C Relative Humidity 70% - +5%		
Parameter	Specifications	Months		
		INITIAL	3	6
Appearance	Aqueous solution	Conform	Conform	Conform
pH	7.0 – 8.5	8.20	7.9	7.8
Assay	90-110%	98.8%	96.2%	94.0%

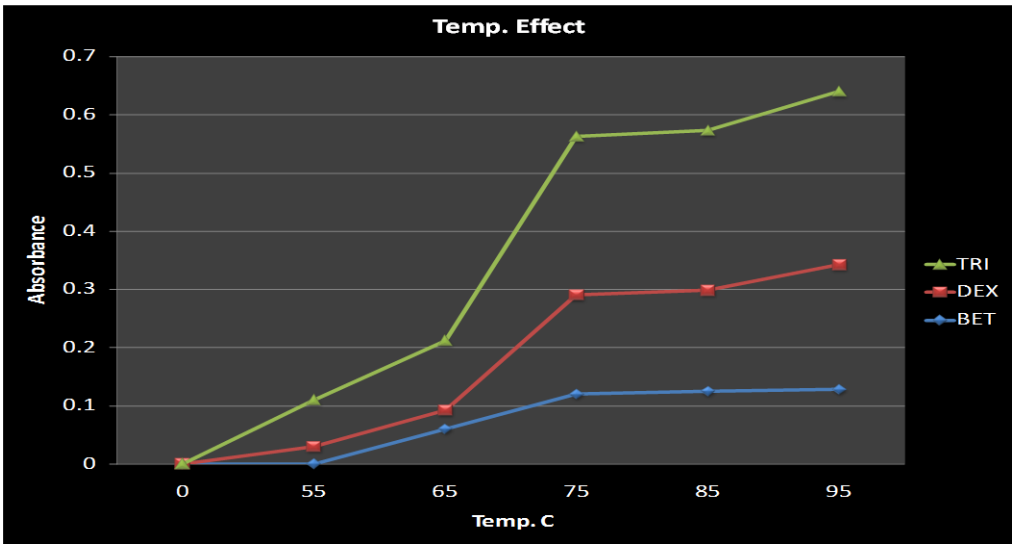


Figure 1: Optimization of Temperature

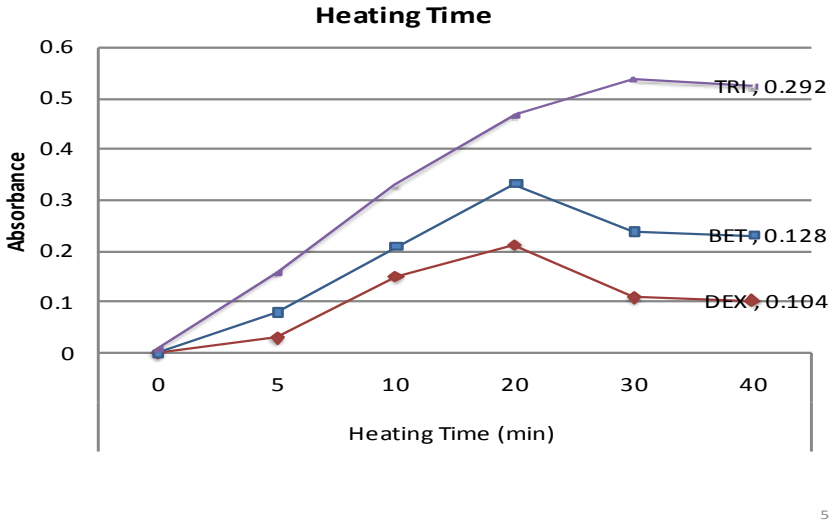


Figure 2: Optimization of Heating Time

CONCLUSION

Different analytical methods and techniques were developed for the determination of glucocorticosteroids Betamethasone, Dexamethasone, Triamcinolone based on simplicity, sensitivity, selectivity, low cost as well as safe. For this purpose, Spectroscopic techniques were employed. The proposed methods based on uv-visible spectrophotometric technique for preferred glucocorticosteroid determination having many advantages over other analytical methods due to its rapidity, lower cost, environmental safety and better sensitivity.

The method can be successfully employed for Betamethasone, Dexamethasone, Triamcinolone; quantification in all types of pharmaceutical preparations. In another approach method was developed for determining Betamethasone, Dexamethasone, Triamcinolone is more superior to reported spectrophotometric methods due to its better sensitivity, selectivity, broader linear working and lower detection limits. Application of the developed method for quantification of Betamethasone, Dexamethasone, Triamcinolone, with good recovery and lower standard deviation proved its suitability for analysis of Betamethasone, Dexamethasone, Triamcinolone, in pharmaceutical preparations. In conclusion, we have developed a simple new method in which 2,4 dinitrophenylhydrzine was used first time for derivatization of Betamethasone, Dexamethasone, Triamcinolone, analyze on double beam spectrophotometer method for the determination purpose. Derivatization was carried out by heating reagent at 100 ^{o}C for 20-30 minutes with using optimized buffer solutions pH 3,6,10, the beer's law acceptance and the calculated molar absorptivity respectively were for BET, DEX, TRI, 2461.4, 965.5, 6142.6 L mole $^{-1}$ cm^{-1}. Under optimized condition by application on pharmaceutical preparations the quantities were found with RSD .039, 0.348, 0.389. The stability was performed under accelerating condition means increased humidity and temperature. Along with other physical parameters observations, was according to the specifications of pharmacopeias and accelerating stability, RSD was observed results were like on an average was 1.84-4.56.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

All authors read and approved the final manuscript.

Authors' Information

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