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"DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC ASSAY METHOD OF FEBUXOSTAT IN BULK AND DOSAGE FORM."

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ABSTRACT

A simple and cost effective uv spectrophotometric method is discribed for the determination of febuxostat in bulk drug and in pharmaceutical formulation. The drug was soluble in 0.1N KOH so it was selected as the solvent system for the drug. This insure adequate drug solubility and maximum assay sensitivity. The linearity range for febuxostat at its wavelength of detection of 287nm was obtained as 2-10µg/ml. The linear regression equation obtained by least square regression method, were, Y=0.005x+0.01,where Y is the absorbance and X is the concentration(in µg/ml) of pure drug solution. The absorbance was found to increase linearly with increasing concentration of febuxostat, showing correlation coefficient value of 1. The validity of the described procedure was assessed. Statistical analysis of the result shows high accuracy and good precision. The proposed method was successfully applied to the determination of febuxostat in pharmaceutical formulations without any interference from common excipients. The proposed method was validated as per the ICH guidelines for linearity, accuracy, precision and robustness. This method can be employed for routine quality control analysis of febuxostat in tablet dosage forms. This method can be used for bioanalysis of febuxostat.

KEYWORDS: UV spectroscopy, Febuxostat, ICH guidelines.

INTRODUCTION[9,10,3]

Antidepressants work by balancing brain neurotransmitters level to ease depression.

Classification of antidepressants & their side effects

1) Tricyclic antidepressants (TCAs)

e.g.- Amitriptyline, Imipramine.

Side effect - Dizziness, headache, sweating, tremor palpitation, dry mouth, etc.

2) Tetracyclic antidepressants

e.g.- Mianserin, Maprotiline

Side effect - constipation, difficulty passing urine, orthostatic hypotension, etc.

3) Selective serotonin re-uptake inhibitors (SSRIs)

e.g.- Fluoxetine, Paroxetine, Sertraline, Fluvoxamine

Side effect- headache, sweating, sexual dysfunction and weight loss, etc.

4) Serotonin and norepinephrine re-uptake inhibitors (SNRIs)

e.g- Venlafaxine/ Venlafaxine XR, Duloxetine.

Side effect- May cause hypertension at high doses.

5) Serotonin receptor modulators (SRMs)

e.g.- Mirtazapine.

Side effect- tremor, headache, constipation, weight gain, hypotension, etc.

6) Monoamine oxidase inhibitors (MAOIs)

e.g- Moclobemide, Phenelzine

Side effects- Dizziness, headache, nervousness, gastrointestinal disturbance, etc.

7) Lithium Salts

e.g- Lithium carbonate.

Side effects- Bitter taste, dry mouth, tremor, polyuria, fatigue and weight gain, etc.

Indications

Mainly relieve the symptoms of depression or bipolar disorder such as depressed mood, worthlessness, lack of motivation or concentration. Antidepressants are also used for anxiety disorder, phobic disorder (such as social phobia or agoraphobia), panic attack, obsessive-

compulsive disorder, bulimia nervosa, nocturnal enuresis, chronic pain, neuropathic pain and post-traumatic stress disorder.

Mechanism of action of antidepressants

According to the monoamine hypothesis of depression postulates a deficiency in serotonin or norepinephrine neurotransmission in the brain. Most of the currently used antidepressants work by slowing the removal of both norepinephrine and serotonin from the brain, thus increasing the availability of these neurotransmitters. As a result, they are efficacious for patients whose depression is caused by the imbalance of either norepinephrine or serotonin

Cautions when taking antidepressants

1) Patients with or have a history of suicidal behavior should avoid taking SSRIs.

2) Do not expect the antidepressants to work right away. It usually takes about 2 to 3 weeks for the antidepressants to start working.

3) To avoid side effects. Do not drink alcohol when taking these medications.

DO's	**DON'Ts**
1) Follow your doctor's direction. 2) Pay attention to the dosage, usage, precaution and side effects of your medications. 3) Read the prescription label carefully. 4) Understand the method of administration. 5) Store your medication properly. 6) Take your medication at the same time of day. 7) Complete the prescribed course unless otherwise directed	1) Change the dosage of your medication on your own. 2) Stop taking your medication except on your doctor's advice. 3) Drink alcohol with your medication. 4) Put your medication in other bottle. 5) Mix 2 medications in one bottle. 6) Take other medication unless directed by your doctor.

UV Spectroscopy methods

It is the branch of science dealing with the study of interaction between electromagnetic radiation and matter. It is a most powerful tool available for the study of atomic and molecular structures and is used in the analysis of wide range of samples.

UV spectroscopy shows absorbance in range200-400 nm.

Beer-lambert's law

When beam of light is passed through a transparent cell containing a solution of unabsorbing substance, reduction of the intensity of light may occur. Mathematically, beer-lamberts law is expressed as

A=a b c

Where, A= absorbance or optical density

a= absorptivity or extinction coefficient

b= path length of radiation through sample(cm)

c= concentration of solute in solution.

Both b and a are constant so a is directly proportional to the concentration c.

When c is in gm/100ml, then the constant is called A(1%,1cm)

$$A=A1\%/1cm*bc$$

Quantification of medicinal substance using spectrophotometer may carried out by preparing solution in transparent solvent and measuring its absorbance at suitable wavelength. The wavelength normally selected is wavelength of maximum absorption (λ_{max}). Ideally concentration should be adjusted to give an absorbance of approximately 0.9, around which the accuracy and precision of the measurements are optimal.

The concentration of the substances in the sample is calculated from the proportional relationship that exists between absorbance and concentration.

$$C_{test}=(A_{test}xC_{std})/A_{std}$$

Where $C_{test}$and $C_{std}$are the concentrations in the sample and standard solutions respectively and $A_{test}$and $A_{std}$are the absorbances of the sample and standard solutions respectively. For assay of substances in multi component samples by spectrophotometer; the following methods are being used routinely, which includes;

1. Simultaneous equation method
2. Derivative spectrophotometric method
3. Absorbance ratio method (Q-Absorbance method)
4. Different spectrophotometry
5. Solvent extraction method[1,5]

Method validation

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

Analytical methods need to be validated or revalidated

1) Before their introduction into routine use;
2) Whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and
3) Whenever the method is changed and the change is outside the original scope of the method.

ICH Guidelines for analytical procedure and validation

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formula for the calculation, etc.

Types of Analytical Procedures to be Validated

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

- Identification tests;
- Quantitative tests for impurities' content;
- Limit tests for the control of impurities;
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below:

1. Accuracy
2. Precision
3. Repeatability
4. Intermediate Precision
5. Specificity
6. Detection Limit
7. Quantitation Limit
8. Linearity
9. Range

MATERIALS AND METHOD

Solvent: 0.1N KOH, DMSO, 0.1N NaOH, Methanol**.**

Grade of solvent: AR grade

Water: distilled water

Working standard: Febuxostat

BATCH NO: OP-FAB/10/017.

MFG DATE: OCT 2012.

A.R.No: OP-FEB 017/12

Formulation: FEBUBEST*-40 tablet (40mg tablet),

BATCH No. FCA2G4A4.

MFG.DATE: JUL.2014.

EXP.DATE: JUN.2016.

MFG BY: INDOCO REMEDIES LTD

Table: List of Equipments

Sr. No.	Name of Equipments	Manufacture
1	Electronic balance	Shimadzu BL220H
2	Ultrasonicator	Bio-technics india
3	UV Visible spectrophotometer	Shimadzu UV-1800, Japan Systronics AU2701

Method Development[1,2,3,4,6,8]

Solubility profile

The febuxostat was found to be soluble in DMSO, 0.1N NaOH, 0.1N KOH, sparingly soluble in methanol.

Trial: 1

Solvent: DMSO (dimethyl-sulfoxide)

It was observed that the clear solution of 1 mg/ml of febuxostat is not formed in DMSO not seen clear peak at its λ max hence I was decided to not to use this solvent.

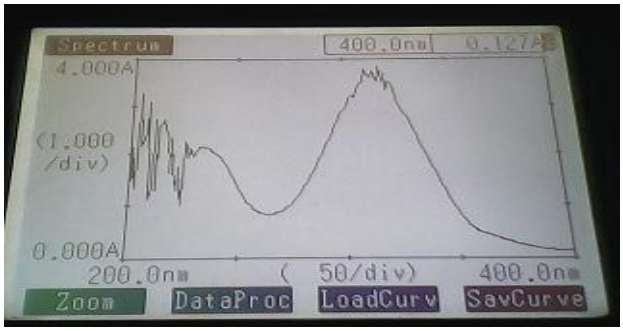


Figure 1: UV spectrum of febuxostat in DMSO

Trial: 2

Solvent: Methanol

It was observed that the clear solution of 1mg/ml of febuxostst is not formed in Methanol not even clear peak at its λ max hence, I was decided to not use this solvent.

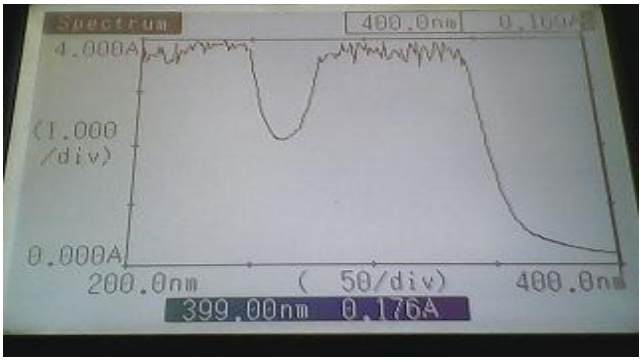


Figure 2: UV spectrum of febuxostat in methanol

Trial: 3

Solvent: 0.1N NaOH

It was seen that 1mg/ml clear solution in 0.1N NaOH formed but should not got the clear peak at its λ max. so I was decided not to use this solvent.

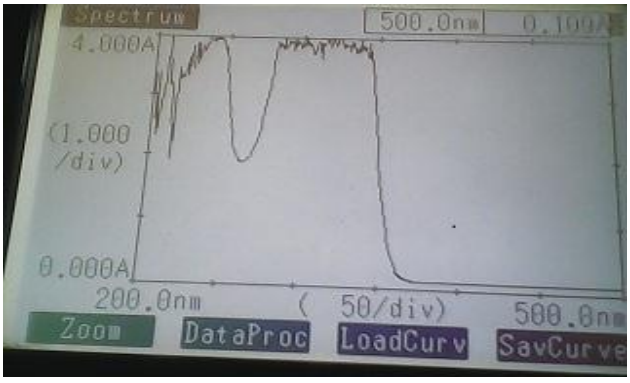


Figure 3: UV spectrum of febuxostat in 0.1 N NaOH

Trial: 4

Solvent: KOH (potassium hydroxide)

It was seen that 1 mg/ml clear solution in 0.1N KOH form and seen the clear peak at its λ max. So I was decided to use this solvent.

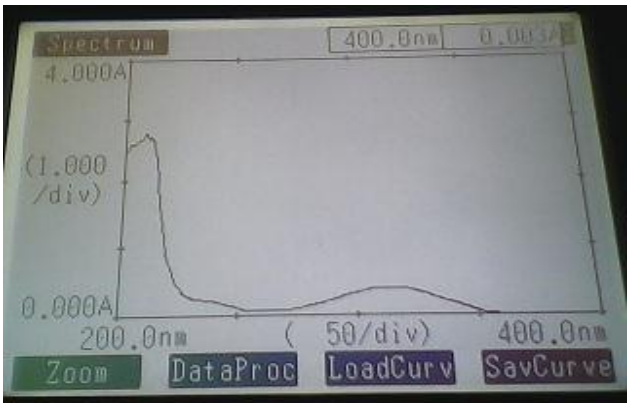


Figure 4: UV spectrum of febuxostat in 0.1N KOH.

Standard solution of Febuxostat

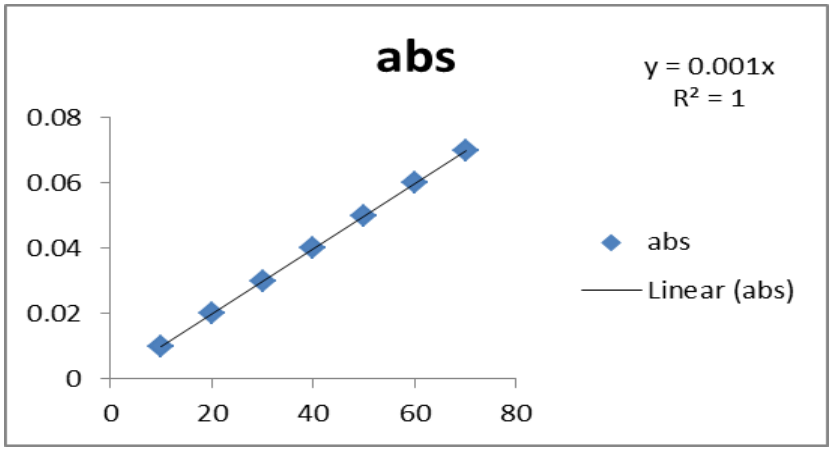
Standard stock solution (primary) was prepared by dissolving 10 mg of Febuxostat in 10 ml of 0.1 N KOH to get concentration of 1mg/ml (1000µg/ml) and was stored at + 4ºC during the study. Secondary stock solution was prepared daily by diluting 1ml of the primary stock solution to final volume of 10 ml using 0.1 N KOH to get concentration of 0.1mg/ml (100µg/ml).

Preparation of calibration standard solutions

Suitable aliquots of the secondary standard solution of Febuxostat (10–70 ml) were transferred to a series of calibrated 100 ml standard volumetric flasks and the volume was made up to the mark with 0.1 N KOH.(table 1&2) (fig.6).

Scanning and determination of λmax

In order to ascertain the wavelength of maximum absorption (λmax) of the drug, qualitative solution of the drug was prepared in 0.1 N KOH and scanned by using UV spectrophotometer within the wavelength region of 200 – 400 nm against 0.1N KOH as blank. The calibration curve was constructed for absorbance versus concentration of Febuxostat. The resulting spectrum was shown in Figure, and the absorption curve showed characteristic absorption maxima at 287nm for Febuxostat.(fig.5).



Calibration curve of febuxostat

Ctest = Atest/Astd × Cstd

= 0.059/0.06 × 10

= 98%

Method Validation[1,2,5,6,7]

1. Linearity and range

Under the experimental condition, the calibration graphs of the absorbance versus concentration were found to be linear over the range of 2-10 µg/ml for proposed method. The statistical analysis of data obtained for the estimation of febuxostat in pure solution indicated high level of accuracy for the proposed methods as evidenced by the low values of standard deviation and coefficient of variation (table 3&4).

The linear regression equation obtained was Y=0.005x+0.01, where Y is the absorbance and X is the concentration (in µg/ml) of pure drug solution (fig.7) Linearity scatter of points for the two drugs by the proposed methods were demonstrated from the highly significant correlation coefficient value.

2. Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of bulk samples of febuxostat to 5 µg/ml so that overall concentration will be within the linearity range. The accuracy was expressed in terms of percent recovery. The mean of percentage recovery values varied from 100.01 to 100.35 (Table5), whereas in Paramdeep et al., method7 the range was 99.23 –100.58. The statistical analysis of data obtained for the estimation of febuxostat indicates a high level of

accuracy for the proposed method as evidenced by the low values of standard deviation and relative standard deviation.

FORMULA OF % RECOVERY

%Recovery= (amount recovered/amount introduced) ×100

3. Precision

The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. Two different concentrations of febuxostat in the linear range (0 .6 ,3 and12 µg/ml) were analyzed in five independent series in the same day (intra-day precision) and in six consecutive days (inter-day precision) and results were given in (Table 6&7). The % RSD values of intra-day and interday studied for the proposed method were in the ranges 0.857 – 2.16 and 2.337 – 0.50 respectively compared to 2.387 – 0.48 and 2.397 – 0.47 respectively .This concludes that the precision of the current method was satisfactory.

4. Detection of LOD and LOQ

For determination of sensitivity of the proposed method, LOD and LOQ were calculated. Based on the signal to noise ratio they were quantified. The lowest detectable concentration of the analyte by the method is LOD where as the minimum quantifiable concentration is LOQ. LOD and LOQ for febuxostat were calculated according to the ICH guidelines by using S (relative standard deviation of the response) and ó (slope of the calibration curve).. These results indicate that the present proposed method was sensitive to detect and quantify.

$$LOD=3.3\times\sigma/S=0.02\mu g/ml \text{ and}$$

$$LOQ=10\times\sigma/S=0.07\mu g/ml$$

5. Ruggedness

The ruggedness of the proposed method was evaluated by applying the developed procedures to assay of 10µg/ml of febuxostat using the same instrument by two different analysts under the same optimized conditions at different days. The obtained results were found to reproducible, since there was no significant difference between analysts. thus, the proposed methods could be considered rugged (table 8).

6. Robustness

The robustness was done by making small changes in the optimized method like change in three different λ max(286,287,288).(table 9).

7. Analysis of pharmaceutical formulations

Ten Febuxostat tablets were taken and the average weight was determined. The tablets were ground and mixed well. The powder of the sample equivalent to 10 mg of Febuxostat was accurately weighed and transferred into a 10 ml volumetric flask. About 7 ml of diluents was added, sonicated to dissolve it completely and made the volume up to the mark with diluent. Mixed well and filtered through Whatmann filter paper. An aliquot equivalent to 20 mg of the sample was pipetted into a 10 ml volumetric flask and made up to the mark after filtration. From the absorbance value, the drug content per tablet (on an average weight basis) was calculated Good recovery values of drugs shows that the proposed method can be successfully applied to the determination of febuxostat in pharmaceutical formulations without any interference from common excipients.(table 10).

RESULT AND OBSERVATION

This UV method is applicable for assay of febuxostat from drug formulation.

Determination of λ max of febuxostat

The absorption maxima of pure drug febuxostat was found to be 3.887 at 287 nm in 0.1N KOH.

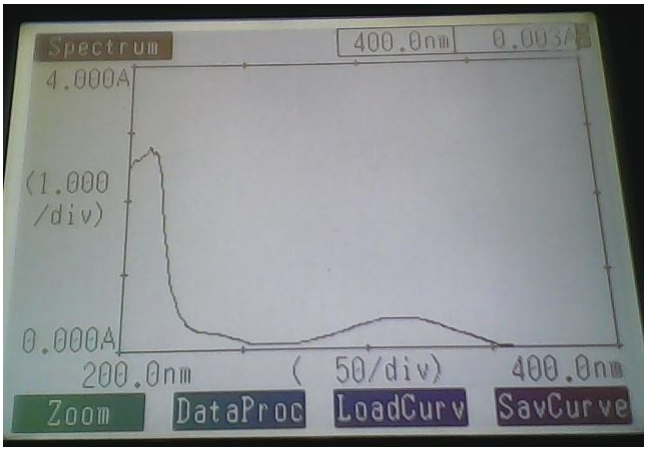


Figure 5: The λmax of febuxostat in 0.1N KOH Calibration curve febuxostat in 0.1N KOH

Calibration curve of febuxostat was found to be in linear in the range of 10-70 µg/ml in 0.1N KOH with correlation coefficient 1. Concentration verses absorbance data is given in table and graphically presented in figure

Table: 1 Observation for calibration curve of febuxostat

Sr. No.	Concentration (µg/ml)	Absorbance (A°)
1	10	0.01
2	20	0.02
3	30	0.03
4	40	0.04
5	50	0.05
6	60	0.06
7	70	0.07

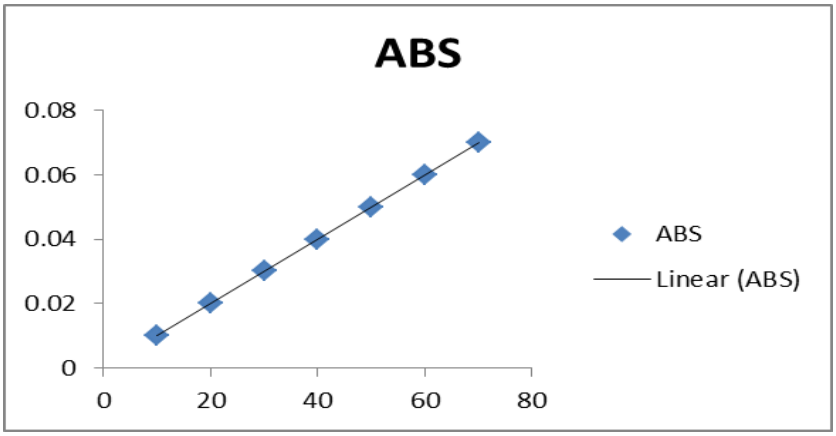


Figure 6: Calibration curve of febuxostat

Table 2: Calibration curve statistics

Sr. No.	Parameter	Observation
1	λmax	287nm
2	Slope	0.001x
3	Intercept	0
4	Coefficient of correlation	1

From the calibration curve slope and intercept (see table) was determined and following equation was used for further calculations:

$$Y = (m) \times (x) + C$$

Where, Y is absorbance, x is concentration, m is slope, C is intercept.

Assay of tablet formulation

$$C_{test} = A_{test} / A_{std} \times C_{std}$$

$$C_{test} = 0.059$$

Cp = 0.06

% purity = Ct/Cp

= 0.059/0.06

= 98 %

Validation method

1) Linearity and range of febuxostat

Table 3: Linearity table of febuxostat in working standard

Concentration (µg/ml)	Mean absorbance	Std error	%CV
2	0.02	0.001	0.05263
4	0.03	0.001	0.04014
6	0.04	0.001	0.03882
8	0.05	0.001	0.03077
10	0.06	0.001	0.02548

Table 4: Analysis of data for the estimation of febuxostat from standard solution

Sr. No.	Parameter	Observation
1	Concentration range	2-10µg/ml
2	Slope	0.005x
3	Intercept	0.01
4	Coefficient of correlation	1

2) Recovery of febuxostat using the proposed uv method

Table 5: Recovery reading

Sample ID	Concentration(µg/ml) Pure drug	Formulation	% Recovery	Statistical Analysis
S1:80%	4	5	99.5	Mean=100.58 SD=1.24 %RSD=1.23
S2:80%	4	5	99.2	
S3:80%	4	5	100.7	
S4:80%	4	5	100.3	
S5:80%	4	5	101.2	
S6:80%	4	5	102.6	
S7:100%	5	5	99.7	Mean=99.83 SD=0.44 %RSD=0.44
S8:100%	5	5	99.2	
S9:100%	5	5	100.1	
S10:100%	5	5	100.3	
S11:100%	5	5	100.1	
S12:100%	5	5	98.2	
S13:120%	6	5	98.9	Mean=99.23 SD=1.01 %RSD=1.02
S14:120%	6	5	98.4	
S15:120%	6	5	99.1	
S16:120%	6	5	99.9	
S17:120%	6	5	99.2	
S18:120%	6	5	100.9	

3) PRECISION

Table 6: INTRADAY PRECISION READING (n=5)

	Conc.(µg/ml)	Mean+_SD	%RSD
Day 1		0.857+_1.852806	2.16%
Day 2	0.6	0.859+_1.851911	2.15%
Day 3		0.860+_1.851464	2.14%
Day 1		2.337+_1.19093	0.50%
Day 2	3	2.387+_1.1685	0.48%
Day 3		2.397+_1.1640	0.48%
Day 1		2.387+_1.1685	0.48%
Day 2	12	2.400+_1.1627	0.47%
Day 3		2.420+_1.153811	0.46%

Table 7: INTERDAY PRECISION READING (n=5)

	Conc.(µg/ml)	Mean+_SD	%RSD
Day 1	0.6	0.857+_1.852806	2.16%
Day 2	3	2.337+_1.19093	0.50%
Day 3	12	2.387+_1.1685	0.48%

4) DETECTION LIMIT &QUANTITATION LI

$$LOD=3.3\sigma/s$$

$$=3.3\times1.8528/2601$$

$$=0.02\mu g/ml$$

$$LOQ=10\sigma/s$$

$$=10\times1.8528/2601$$

$$=0.07\mu g/ml$$

5) RUGGEDNESS

Table 8: RUGGEDNESS DATA AT 10mg/ml BY TWO ANALYSTS AT DIFFERENT DAYS

Test concentration	Amount recovered (µg/ml)Analyst 1	Amount recovered (µg/ml) Analyst 2
10µg/ml	4.79	4.78
	4.82	4.81
	4.86	4.85
Mean	4.89	4.88
	4.92	4.91
	4.796	4.846
SD	0.0522	0.0522
%RSD	0.0108	0.0107

6) ROBUSTNESS

Table 9: Robustness of the method

λ max	Mean	SD	%RSD
286	0.083	0.00816	0.098
287	0.081	0.00753	0.092
288	0.081	0.00753	0.092

7) ANALYSIS OF PHARMACEUTICAL FORMULATION

Table 10: Analysis of pharmaceutical formulation

Formulation	Labelled amount(mg)	Uv spectro- Amount recovered(mg)	Metric %drug recovered	Method %RSD
Febubest-40	40mg	39.80+_0.0103	99.521	1.032

DISCUSION

In this present study an attempt has been made to develop UV spectrophotometric method for the determination of febuxostat in pure & tablet dosage form. The result obtained were reproducible & reliable. The validity & accuracy of the method were evident from the statistical & analytical parameters obtained. Therefore, it included that the method is suitable for application on routine quality control analysis of pharmaceutical preparation.

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