SPECTROPHOTOMETRIC DETERMINATION OF DRUGS AND PHARMACEUTICALS USING KMNO₄ AS OXIDANT AND AMARANTH DYE AS ANALYTICAL REAGENT

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ABSTRACT
Simple, sensitive and accurate methods for determination of four drugs viz., Imatinib Mesylate, Oseltamivir Phosphate, Venlafaxine Hydrochloride and Dextrometharphan HBr have been developed. The methods depend upon the oxidation of the drug by a known excess Ceric Ammonium Sulphate in sulphuric acid medium and subsequent determination of unreacted Ce (IV) using Amaranth dye. The methods have been validated in terms of LOD, LOQ, precision accuracy, %RSD, robustness and ruggedness. Factors affecting the absorbance viz., concentration of H₂SO₄ and time of reaction are optimized. The effect of excipients has also been studied and found to have no effect. The calibration curves are found useful for determination of pure drug and can be applied to pharmaceuticals in bulk drug and pharmaceutical industries.

KEYWORDS: Spectrophotometry, Drugs, Ceric Ammonium Sulphate, Amaranth Dye, Validation.

1. INTRODUCTION
Imatinib mesylate (ITM) is a most frequently prescribed cancer medication drug to treat leukemia and gastrointestinal tumors. It operates by inhibiting proteins associated with cancer cell growth in order to relieve symptoms, prevent the spread of cancer cells and aid other treatments. The drug is designed to inhibit tyrosine kinases such as Bcr-Abl and is used in the treatment of chronic myeloid leukemia (CML) and gastrointestinal stromal tumor.[1] Imatinib
mesylate was approved by the US Food and Drug Administration (FDA) to treat a rare cancer called chronic myeloid leukemia (CML).\[2\] Imatinib mesylate\[3\] is chemically known as 4-4\(((4\text{-methyl-1-}

\text{piperazinyl})\text{ methyl})\text{-N-}[4\text{-methyl-3-}\[4\text{-}\text{(3-pyridinyl)-2-pyrimidinyl}]\text{ amino}\[phenyl]} – \text{ benzamide mono methane sulfonate} (fig.1) with experiential formula C\text{29}H\text{31}N\text{7}O\text{•CH}_3\text{SO}_3\text{H}. Literature survey reveals that several analytical methods like UV-spectrophotometric\[4\], HPLC\[5\], Charge Transfer complex\[6\], RP-HPLC\[7\] methods are reported for estimation of Imatinib mesylate in bulk drug, formulations, pure active pharmaceutical ingredient and tablet dosage form.

Oseltamivir is an ester prodrug. It comes under the category of drugs called neuraminidase inhibitors.\[8\] It works by stopping the spread of flu virus in the body. It has an antiviral activity. Its active metabolite selectively blocks the viral surface enzyme neuraminidase thereby preventing the release of virus particles from infected cells. It is active against influenza A and B virus and is the drug of choice for treatment of swine flu. Oseltamivir\[3,9\] is chemically known as (3\text{R}, 4\text{R}, 5\text{S})\text{-4-(Acetylamino)-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid ethylester.} (fig.2). Literature survey reveals that there are some reported analytical methods like UV –Spectrophotometry\[10\], HPLC\[11\], RP-HPLC\[12\], HPTLC\[13\], Colorimetric and liquid chromatographic\[14\] methods are reported for estimation of Oseltamivir Phosphate in bulk drug, formulations, pure active pharmaceutical ingredient and tablet dosage form.

Venlafaxine (VEN) ia a commonly prescribed antidepressant drug. It is a third generation antidepressant. It inhibits the reuptake of serotonin, nor epinephrine and to a lesser extent dopamine. It is prescribed for the treatment of clinical depression and anxiety disorders, social phobia, panic disorder, and vasomotor symptoms.\[15,16\] It is chemically known as 1\text{-}[2\text{-}(\text{dimethylamino})\text{-1-}((4\text{-methoxyphenyl})\text{ ethyl}]\text{ cyclohexan-1-ol.} (fig.3). The method has been validated in and according to ICH guidelines.\[17,18\] VEN is metabolized by the liver enzyme cytochrome 450 2D6 and 2C19 into several metabolites. The activity of these enzymes is influenced by genetic differences which can cause large inter individual variation in the drug metabolism and clearance. The method has been validated in and according to ICH guidelines.\[3,4\] Literature survey revealed that most of the HPLC\[19,20\] methods used with detectors such as mass-spectrometry, fluorimetry, Electrospray mass spectrometric techniques all these methods have high sensitivity, but most of them highly expensive and are not easily available in quality control laboratories. Only few UV spectrophotometric
methods\textsuperscript{[21,22]} are reported. So author’s objective is to develop accurate, simple, sensitive, new UV spectrophotometric method which is free from extraction techniques and highly sensitive.

Dextromethorphan (DXM) drug belongs to the class of morphinan with sedative and disassociative properties. Dextromethorphan is the dextrorotatory enantiomer of levomethorphan, which is the methyl ether of levorphanol, both opioid analgesics. Dextromethorphan is commonly available as the monohydrated hydrobromide salt. It is chemically known as (+)-3-methoxy-17-methyl-9\textalpha, 13\textalpha, 14\textalpha-morphinan hydrobromide monohydrate (fig.4). It is an anti tussive (cough suppressant) drug used for the relief of non-productive cough, it has a central action on the cough centre in the medulla. Dextromethorphan is rapidly absorbed from the gastrointestinal tract and converted into the active metabolite dextrorphan in the liver by the cytochrome P450 enzyme CYP2D6, where it enters the blood stream and crosses the blood–brain barrier. Literature survey reveals that there are some reported analytical methods like UV –Spectrophotometry\textsuperscript{[23]}, HPLC\textsuperscript{[24]}, RP-HPLC\textsuperscript{[25]} Spectrophotometric and liquid chromatographic\textsuperscript{[26]} methods are reported for estimation of Oseltamivir Phosphate in bulk drug, formulations, pure active pharmaceutical ingredient and tablet dosage.

Although much work has been published on the quantification of the above drugs but the simplest method using oxidative spectrophotometry has not been reported yet. In the present communication we report the oxidation of drug by KMnO$_4$ - Amaranth dye couple to report the quantification of drug by KMnO$_4$ as oxidant and Amaranth dye as analytical reagent.

**STRUCTURE OF DRUGS**

![Fig 1. Imatinib Mesylate](image1)

![Fig 2. Oseltamivir Phosphate](image2)
2. ABOUT THE METHOD
Cerium (IV) is a good oxidizing agent like KMnO₄, K₂Cr₂O₇ etc., it has been used for quantitative determination of drugs based on the oxidation of drugs. The spectrophotometric methods involved addition of excess Ce(IV) and unreacted cerium is estimated by suitable dyes, which should be oxidized by cerium viz., Indigo Carmine, Methyl Orange, Safranin-O and Xylene cyanol.

Amaranth dye is suitable for estimation of unreacted Ce(IV) absorbance at 523 nm. Cerium(IV) is a strong oxidizing agent due to its highest oxidation potential \((E_0 = 1.44 \text{ V})\).

3. EXPERIMENTAL
3.1 Instrumentation
The UV-VIS spectra of the study have been recorded on ELICO 210 double beam Spectrophotometer, Thermo Nicolet 1000 and also on ELICO 159 UV-VIS single beam spectrophotometers using quartz cells of 10 mm path length. A Dhona 200 single pan electrical balance is used for weighing the samples.

3.2 Materials and methods All reagents used were of analytical-reagent grade and distilled water was used throughout the investigation.

3.2.1 Cerium (IV) solution was prepared by dissolving 750 mg of Cerium (IV) sulphate (CeSO₄·2H₂O, 99.9 % pure) (Merck, Mumbai, India) in 2 N H₂SO₄ with the aid of heat, filtered using glass wool, and diluted to 250 ml with the same acid and standardized. Cerium is standardized by ferrous ammonium sulphate and ferroin indicator. The solution was then diluted appropriately with 2 N H₂SO₄ to get working concentrations of \(4.0 \times 10^{-3} \text{ M} \) (0.25%).
3.2.2 **Amaranth dye**: Aqueous solution of 0.8x10^{-3} \textit{M} of Amaranth dye was prepared by dissolving an appropriate weight of 0.0483 grams in 100 ml by distilled water.

3.2.3 **Sulphuric acid**: Prepared by diluting the concentrated acid (Merck, Mumbai, India, Sp. gr. 1.84, 98.0 %) with water appropriately to get 2 \textit{N} acid.

3.2.4 **Preparation of drug solution** Standard drug solution (200 \textmu g ml^{-1}) was prepared by dissolving accurately weighed 20 mg drug with suitable solvent to the mark in 100 ml standard flask. The stock solutions of ITM, OSP, VEN and DEX were further diluted with the same solvent to obtain working concentrations.

4. **PROCEDURE**

Aliquots containing 3-21 \textmu g ml^{-1} (ITM), 3.5-24.5 \textmu g ml^{-1} (OSP), 3.2-22.4 \textmu g ml^{-1} (VEN), 4-28 \textmu g ml^{-1} (DEX) of drug were transferred into a series of 10 ml standard flasks using a micro burette. To this, 1 mL of CAS was added followed by 1 mL of 2\textit{N} H_2SO_4 and contents were shaken well. After 15 minutes, 1mL of 0.02\% of amaranth added to the contents. Then contents were shaken well and diluted with double distilled water up to the mark. The absorbance of each solution was measured at 523 nm against the corresponding reagent blank.

5. **ASSAY OF PURE DRUG SAMPLE**

To test the accuracy and precision of the methods developed, pure sample solutions containing drug in the Beer’s Law limit were chosen. For this study 3-21 \textmu g mL^{-1} of ITM, 3.5-24.5 \textmu g mL^{-1} of OSP, 3.2-22.4 \textmu g mL^{-1} of VEN, 4-28 \textmu g mL^{-1} of DEX. To each of the solution 1 mL of 250 \textmu g mL^{-1} of cerium, 1 ml of 2 \textit{N} of H_2SO_4 were added and the un reacted cerium is analyzed as described above using amaranth dye. Calibration curves were constructed for all the drugs by plotting the absorbance versus the concentration of drugs. The absorbance data was collected for six replicate experiments and absorbance to concentration ratio called the relative response was determined. The relative responses between 95\% to 105\% of average are only considered for construction of the Calibration curves.
5.1 Procedure For Assay Of Pure Drug
Sample solutions of each drug in the beer’s law limits were chosen and recovery experiments were performed to check the accuracy and precision. The concentration chosen and % of recovery are tabulated in table2, for this purpose standard deviation method also adapted. Excellent recovery and %RSD being less than 2 speaks about the precision and accuracy of the method.

6. PROCEDURE FOR ANALYSIS OF TABLETS
6.1 Imatinib Mesylate
For the analysis of pharmaceutical formulations two tablets (veenat – 100mg) were weighed accurately and grounded. A quantity equivalent to 10mg of Imatinib mesylate was weighed accurately, transferred into a 100 mL calibrated flask and the volume was finally diluted to the mark with double distilled water, mixed well and filtered using a Whatman No. 42 filter paper. It was used as stock sample solution and was further diluted with water to get working standard solution.

6.2 Oseltamivir Phosphate
Two Capsules (Tamiflu, 75mg) were opened and carefully separated the powder. A quantity equivalent to 10 mg of Oseltamivir Phosphate was weighed accurately, transferred into a 100 mL calibrated flask and the volume was finally diluted to the mark with double distilled water, mixed well and filtered using a Whatman No. 42 filter paper. It was used as stock sample solution and was further diluted with water to get working standard solution.
6.3 Venlafaxine Hydrochloride
Six tablets (Venlafaxine Tablets, USP 25mg) were weighed accurately and crushed to a fine powder and the powder equivalent to 10g of Venlafaxine Hydrochloride was weighed accurately and transferred to 100 ml volumetric flask, dissolved in sufficient quantity of methanol, sonicated for 10 min and the volume was finally diluted to the mark with methanol. This solution was mixed well and filtered through Whatman filter paper No. 42. It was used as stock sample solution and was further diluted with the same solvent to get working standard solution.

6.4 Dextromethorphan Hydrobromide
Ten tablets (Lastuss-CT-10mg) were crushed to a fine powder and the powder equivalent to 10mg of Dextromethorphan Hydrobromide was weighed accurately and transferred to 100 mL volumetric flask, dissolved in sufficient quantity of methanol, sonicated for 10 min and the volume was finally diluted to the mark with methanol. This solution was mixed well and filtered through Whatman filter paper No. 42. It was used as stock sample solution and was further diluted with the same solvent to get working standard solution.

7. METHOD OF VALIDATION
The each method developed for quantification of drugs has been validated in terms of precision, accuracy, limit of detection, limit of quantification, linearity, selectivity and ruggedness. Absorbance-concentration curves were drawn, fixed time method was used to assess the recovery of the drug. To assess the precision each experiment was repeated at least 6 times and accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery and RSD being less than 2 for each drug demonstrates accuracy and precision of the methods.[Table2].

As mentioned earlier limit of detection is the minimum limit that can be detected but not necessarily quantified is determined for each drug.

LOD is determined from the standard deviation of y-intercepts of regression lines of replicate determinations.

LOD = 3.3 s_a/S

Where s_a = standard deviation of intercept (n=6)
S = slope of linearity plot LOQ the minimum concentration of analyst using calibration curve is also determined. LOQ = 10s_a/S.
Limits of linearity of calibration curves [Fig. 5] are mentioned in the under the title Beer’s law limit. To test the selectivity known excipients of each drug are added to the pure drug sample and recovery experiments were performed. Ruggedness is resistance of method for a small change in variables like instrument, and analyst or both to test the ruggedness of the method absorbance data was collected using 3 different instrument and 2 analysts no significant changes were observed either by change of instrument or analyst hence the method may be taken as robust.

8. FACTORS EFFECTING ABSORBANCE AND SELECTION OF ACID

8.1 Selection of acid: To study the effect of acid, different types of acids were examined (H₂SO₄, H₃PO₄ and CH₃COOH) to achieve maximum yield of Redox reaction. The results indicated that the Sulphuric acid was the preferable acid with Ce (IV) as oxidant.

8.2 Selection of volume of acid and concentration: To study the effect of acid concentration, different concentrations of H₂SO₄ were examined. The reaction was performed in a series of 10 ml volumetric flask containing 12.0 µg mL⁻¹ of the cited drugs, different volumes (0.5–2.5 mL) of 0.5 N, 1.0 N, 1.5 N, 2.0 N, 2.5 N H₂SO₄ and 1 ml of Ce(IV) (4.0x10⁻³ M) were added. After 15 min of time, 1.0 ml of amaranth dye and water added upto the mark. It was found that the maximum absorbance was obtained with 1mL of 2N H₂SO₄. Above this volume, the absorbance decreased therefore, a volume of 1 mL of 2 N H₂SO₄ was used for all measurements.

8.3 Effect of time: In order to obtain the highest and most stable absorbance, the effect of time on the oxidation reaction of drugs were catalyzed by the time periods ranging for 2.5–20 min. the time required to complete the reaction and maximum absorbance was obtained after 15 min.

9. ANALYSIS OF PHARMACEUTICALS

To the test the applicability of the method developed solution of pharmaceutical tablets solutions containing drug in the Beer’s Law limit were chosen. To assess the precision each tablet analysis was repeated at least 6 times and accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery and RSD being less than 2 for each drug demonstrates applicability of the methods for pharmaceutical analysis [Table 3]. The excellent recovery studies indicate that methods developed can be applied to pharmaceutical analysis without hesitation.
10. RESULTS AND DISCUSSION

The ability of cerium (IV) sulphate to oxidize drugs and bleach the color of amaranth dye is the basis of the indirect spectrophotometric method developed here. In this method the drugs were reacted with a measured excess of Cerium (IV) sulphate in acidic medium and the unreacted oxidant was determined by reacting with amaranth followed by absorbance measurement at 523 nm (scheme1). The absorbance increased linearly with increasing concentration of drug, when increasing amounts of each drug were added to a fixed amount of 0.25% of CAS, consumed the latter and there occurred a concomitant fall in its concentration. When fixed amount of the dye was added to decreasing amount of oxidant, an concomitant increase in the concentration of dye resulted. This was observed as a proportional increase in absorbance at the respective \( \lambda_{\text{max}} \) with increasing concentration of each drug. One ml of 2N acid was used in the reaction, as this concentration was found ideal.

Drug + Ce (IV) excess \( \rightarrow \) Drug oxidation product + Ce (III) + Ce (IV) unreacted;

Ce (IV) unreacted + amaranth \( \rightarrow \) oxidation product of amaranth + Unreacted amaranth, measured spectrophotometrically at \( \lambda_{\text{max}} = 523 \) nm.

Tentative reaction scheme for the indirect determination of drug by oxidation with Ce (IV) sulphate

11. ANALYTICAL DATA

A linear correlation was found between absorbance at \( \lambda_{\text{max}} \) and concentration ranges, and sensitivity parameters such as Sandal’s sensitivity, detection limit and quantification limit calculated according to ICH guidelines\(^{[18]}\) are also presented in table 1 and reveal the very high sensitivity of the methods Regression analysis of Beer’s law data using the method of least squares was made to evaluate the slope (b), intercept (a), correlation coefficient (r) and is also given in [Table 1].

Table 1: Analytical and Regression parameters of Spectrophotometric Method

<table>
<thead>
<tr>
<th>Name of drug Property</th>
<th>ITM</th>
<th>OSP</th>
<th>VEN</th>
<th>DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}}, \text{ nm} )</td>
<td>523</td>
<td>523</td>
<td>523</td>
<td>523</td>
</tr>
<tr>
<td>Beer’s law limits (( \mu g \text{ mL}^{-1} ))</td>
<td>3.0-21.0</td>
<td>3.5-24.5</td>
<td>3.2-22.4</td>
<td>4.0-28</td>
</tr>
<tr>
<td>Molar absorptivity</td>
<td>( 2.07 \times 10^4 )</td>
<td>( 1.39 \times 10^4 )</td>
<td>( 1.18 \times 10^4 )</td>
<td>( 1.21 \times 10^4 )</td>
</tr>
<tr>
<td>Sandell’s sensitivity (( \mu g \text{ cm}^2 ))</td>
<td>0.0259</td>
<td>0.0305</td>
<td>0.0270</td>
<td>0.0335</td>
</tr>
<tr>
<td>Variance (( S_a^2 ))</td>
<td>0.00004</td>
<td>0.000002</td>
<td>0.000007</td>
<td>0.000002</td>
</tr>
<tr>
<td>Limit of detection ( \mu g \text{ mL}^{-1} )</td>
<td>0.1808</td>
<td>0.1713</td>
<td>0.0248</td>
<td>0.0156</td>
</tr>
<tr>
<td>Limit of quantification ( \mu g \text{ mL}^{-1} )</td>
<td>0.5481</td>
<td>0.5192</td>
<td>0.0752</td>
<td>0.0474</td>
</tr>
</tbody>
</table>
Regression equation, $Y^{**} = a + bX$

<table>
<thead>
<tr>
<th>Intercept, $(a)$</th>
<th>0.0073</th>
<th>0.0029</th>
<th>0.0044</th>
<th>0.0258</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope, $(b)$</td>
<td>0.0374</td>
<td>0.0312</td>
<td>0.0372</td>
<td>0.0297</td>
</tr>
<tr>
<td>Correlation coefficient, $(r)$</td>
<td>0.9995</td>
<td>0.9998</td>
<td>0.9997</td>
<td>0.9976</td>
</tr>
<tr>
<td>Standard deviation of intercept $(S_a)$</td>
<td>0.0020</td>
<td>0.0016</td>
<td>0.0052</td>
<td>0.0156</td>
</tr>
<tr>
<td>Standard deviation of slope $(S_b)$</td>
<td>0.0015</td>
<td>0.0021</td>
<td>0.0003</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*Limit of determination as the weight in µg per mL of solution, which corresponds to an absorbance of $A = 0.001$ measured in a cuvette of cross-sectional area $1 \text{ cm}^2$ and path length of $1 \text{ cm}$. $Y^{**} = a + bX$, where $Y$ is the absorbance and $X$ concentration of drugs in µg per mL.

12. ACCURACY AND PRECISION

The accuracy and precision of the methods were established by analyzing the pure drug solution at 6 different levels (with working limits). The relative error (%) which is a measure of accuracy & RSD (%) a measure of precision are summarized in Table 2 and reveal the high accuracy and precision of the methods.

Table 2 Determination of accuracy and precision of the methods on pure drug Samples.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Taken (µg/ml)</th>
<th>Found (µg/ml)</th>
<th>error (%)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
<th>Proposed method Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITM</td>
<td>3.0</td>
<td>2.99</td>
<td>0.33</td>
<td>99.66</td>
<td>0.0851</td>
<td>99.75 ± 0.0849</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>6.01</td>
<td>0.17</td>
<td>100.17</td>
<td>99.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>8.98</td>
<td>0.22</td>
<td>100.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OSP</td>
<td>3.5</td>
<td>3.49</td>
<td>0.28</td>
<td>99.71</td>
<td>0.2349</td>
<td>99.98 ± 0.2348</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>7.01</td>
<td>0.14</td>
<td>100.14</td>
<td>100.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.5</td>
<td>10.51</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEN</td>
<td>3.2</td>
<td>3.22</td>
<td>0.62</td>
<td>100.62</td>
<td>0.3877</td>
<td>100.22 ± 0.3885</td>
</tr>
<tr>
<td></td>
<td>6.4</td>
<td>6.39</td>
<td>0.16</td>
<td>99.84</td>
<td>100.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.6</td>
<td>9.62</td>
<td>0.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEX</td>
<td>4.0</td>
<td>4.01</td>
<td>0.25</td>
<td>100.25</td>
<td>0.2295</td>
<td>99.98 ± 0.2294</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>7.99</td>
<td>0.12</td>
<td>99.87</td>
<td>98.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.0</td>
<td>11.98</td>
<td>1.16</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

13. ROBUSTNESS AND RUGGEDNESS

To evaluate the robustness of the methods, volume of Hydrochloric acid was slightly altered. The reaction time (after adding CAS, time varied was $10 \pm 2\text{min}$) and the time after addition of dye is slightly changed. To check the ruggedness, analysis was performed by three different analysts and on three different spectrophotometers by the same analyst.

14. APPLICATION TO FORMULATIONS

The proposed methods were applied to the determination of drugs in tablets. The results in Table 3 showed that the methods are successful for the determination of drugs and that the
excipients in the dosage forms do not interfere. The results are compared to the available validated reported\(^{35-38}\) methods on each drug and the results agree well with the claim and also are in agreement with the results obtained by the literature method. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed methods and the literature method at the 95% confidence level with respect to accuracy and precision.

Recovery experiment was performed via standard addition technique to ascertain the accuracy and validity of the proposed methods. To a fixed and known amount / concentration of drug in tablet powder, pure drug was added at three levels (50, 100 and 150% of the level present in the tablet) and the total was found by the proposed methods. Each experiment was repeated six times and the percent recovery of pure drugs added (Table 3) was within the permissible limits showing the absence interference by the inactive ingredients in the assay.

<table>
<thead>
<tr>
<th>Tablets</th>
<th>Drug in tablet (\mu g) mL(^{-1})</th>
<th>Drug added (\mu g) mL(^{-1})</th>
<th>Total found (\mu g) mL(^{-1})</th>
<th>Error (%)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
<th>Reference method Mean±SD</th>
<th>Proposed method Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Veenat (ITM)</strong></td>
<td>0.50 3.0 6.0 9.0 3.0 0.0 9.0</td>
<td>3.51 6.48 9.52 3.00 5.99 9.01</td>
<td>0.28 0.31 0.21 0.00 0.16 0.11</td>
<td>100.28</td>
<td>0.2255</td>
<td>100.5±0.55 (n=3)</td>
<td>100.021±0.2256</td>
<td></td>
</tr>
<tr>
<td><strong>Oseltimivir Phosphate (OSP)</strong></td>
<td>0.50 7.0 10.5 3.5 0.0 7.0 10.5</td>
<td>3.99 7.52 11.0 3.49 7.01 10.52</td>
<td>0.25 0.27 0.00 0.28 0.14 0.19</td>
<td>100.30 ±0.1 (n=6)</td>
<td>0.2164</td>
<td>100.01 ±0.2163</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Venlafaxine (VEN)</strong></td>
<td>0.50 6.4 9.6 3.2 0.0 6.4 9.6</td>
<td>3.70 6.89 10.2 3.20 6.41 9.61</td>
<td>0.00 0.14 0.09 0.00 0.15 0.10</td>
<td>100.00</td>
<td>0.1051</td>
<td>99.55±0.3 (n=3)</td>
<td>100.03±0.1051</td>
<td></td>
</tr>
<tr>
<td><strong>Lastuss-CT (DEX)</strong></td>
<td>0.50 4.0 8.0 12.0 4.0 0.0 12.0</td>
<td>4.49 8.52 12.5 4.01 7.99 12.01</td>
<td>0.22 0.23 0.00 0.25 0.12 0.08</td>
<td>99.77</td>
<td>0.1892</td>
<td>99.48±0.65 (n=3)</td>
<td>100.03±0.1893</td>
<td></td>
</tr>
</tbody>
</table>
Table 4: F-test and t-test values

<table>
<thead>
<tr>
<th></th>
<th>Veenat (ITM)</th>
<th>Oseltamivir Phosphate (OSP)</th>
<th>Venlafaxine (VEN)</th>
<th>Lastuss-CT (DEX)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-test*</td>
<td>0.302</td>
<td>0.01</td>
<td>0.09</td>
<td>0.4225</td>
</tr>
<tr>
<td></td>
<td>(4.7571)</td>
<td>(4.2839)</td>
<td>(4.7571)</td>
<td>(4.7571)</td>
</tr>
<tr>
<td>t-test**</td>
<td>0.981</td>
<td>1.195</td>
<td>1.092</td>
<td>1.202</td>
</tr>
<tr>
<td></td>
<td>(3.182)</td>
<td>(2.447)</td>
<td>(3.182)</td>
<td>(3.182)</td>
</tr>
</tbody>
</table>

*t- test and **F-test values from literature.

15. CONCLUSION
The present study described the successful development of new, simple, sensitive, selective, accurate and rapid spectrophotometric method for the accurate determination of the above drugs in its pharmaceutical form by using Cerium (IV) sulphate as the oxidizing reagent. There is no interference from additives and excipients. The method thus can be used in the determination of these drugs in pure and pharmaceutical formulations. So, it is the good alternative to the reported methods for the determination of these drugs.

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