SPECTROPHOTOMETRIC DETERMINATION OF DRUGS IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY OXIDATION WITH NBS AS ANALYTICAL REAGENT

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ABSTRACT
A simple, precise and an accurate UV-Visible spectrophotometric method has been developed for the assay of five drugs viz., Olanzapine, Valacyclovir, Dronedarone, Donepezil and Vilazodone using NBS as analytical agent. This method is indirect and is based on the determination of surplus NBS, after allowing the reaction between drug and a measured amount of NBS. The unreacted NBS is determined by the measurement in the decrease in the absorbance of the Rhodamine-B dye. The absorbance was measured at 557nm. The proposed method has been conveniently applied in the analysis of the drug in pure form as well as commercially available form. The results of analysis have been validated for accuracy, linearity, precision, LOD and LOQ.

Keywords: Spectrophotometry, Rhodamine-B, Drugs, Quantification and Validation.

INTRODUCTION
Olanzapine (OLP)
Olanzapine chemically known as 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2, 3-b]15 benzodiazepine, is an atypical antipsychotic agent, also known as second-generation antipsychotic (SGA). Since its introduction in 1996 in over 84 countries, several workers have reported HPLC methods for the determination of OLP in plasma, serum, human breast milk and rat brain. A survey of literature showed HPTLC, UV, Linear voltammetry and LC have reported for the assay of OLP in pharmaceuticals.

Valacyclovir (VAL)
Valacyclovir hydrochloride (VAL) [(S)-2-[(2-amino-6-oxo-6,9-dihydro-3H-purin-9-yl) methoxy]ethyl-2-amino-3-methylbutanoate] is a hydrochloride salt of L-Valyl ester of acyclovir. It is an oral antiviral drug used to treat infections with herpes zoster (shingles), herpes simplex genitalis (genital herpes), and herpes labialis (cold sores). It inhibits the replication of viral DNA. It is a prodrug intended to increase the bioavailability of acyclovir by increasing lipophilicity. Valacyclovir is converted by esterase to active drug acyclovir via hepatic first pass metabolism.

Dronedarone (DDR)
Dronedarone hydrochloride, mainly used for the treatment of cardiac arrhythmias, is chemically N-(2-butyl-3-[3-(dibutylamino) propoxy] benzyol)-1-benzofuran-5-yl) methane-sulfonamide. The drug is approved to be used in patients whose hearts have returned to normal rhythm or who will undergo drug or electric-shock treatment to restore a normal heart beat. HPLC, UV and RP-UPLC are well-known and widely used analytical techniques for the analysis of dronedarone and its pharmaceutical dosage forms.

Donepezil (DPZ)
Donepezil hydrochloride, chemically 2, 3-dihydro-5, 6-dimethoxy-2-[(1-phenylmethyl)-4-piperidinyl] methyl]-1-H-inden-1-one hydrochloride. It is clinically used worldwide for...
patients with mild to severe Alzheimer disease29. The most commonly used techniques for the determination of Donepezil hydrochloride are LC30 and RP-HPLC31 methods.

Vilazodon (VZD)
Vilazodone IUPAC Name is 5-(4-[4-(5-cyano-1H-indol-3-yl)butyl)piperazin-1-yl) benzofuran-2carbox amide. It belongs to the category serotonergic antidepressant. Vilazodone was approved by the FDA for use in the United States to treat major depressive disorder in January 21, 2011. Vilazodone acts as a serotonin reuptake inhibitor and 5-HT1A receptor partial agonist It has negligible affinity for other serotonin receptors such as 5-HT1D, 5-HT2A, and 5-HT2C. There are very few methods reported for the determination of Vilazodone viz. UV33 method, spectrofluorimetric and also an RP-HPLC34 methods were reported for its estimation in bulk and pharmaceutical formulation. The present investigation aims to develop more sensitive, simple, eco friendliness and cost effective methods for the determination of these drugs in pure form and in dosage form.

EXPERIMENTAL
Instrument
All absorbance measurements were recorded on Shimadzu 2600 double beam UV-Visible spectrophotometer as well as on Thermo Nicolet 100 and Elico 159 UV- Visible single beam spectrometers using matched pair of Quartz cells of 10mm path length.

MATERIALS AND REAGENTS
The NBS was supplied by Himedia Laboratories Pvt.Ltd., Mumbai. The Rodamine-B and HCL were supplied by SD fine chem.Ltd. Mumbai. India. These drugs were supplied by MSN Laboratories Ltd. All the reagents used were of analytical reagent grade and distilled water was used throughout the investigation.

STANDARD AND SAMPLE PREPARATION
N-Bromosuccinimide (NBS)
An approximately 0.01m solution was prepared by dissolving 0.1779 grams of NBS in 100ml of distilled water. The solution was kept in an amber colored bottle and was diluted with distilled water approximately to get 70µg/mL of NBS.

Rhodamine-B
A stock solution of Rodamine-B (500 µg/mL) was prepared by dissolving the dye in water. The stock solution was diluted with distilled water to get 50µg/mL of Rodamine-B.

Hydrochloric Acid (HCl)
Concentrated HCL is diluted approximately with water to get 1M acid.

Drugs
Into a 100ml of calibrated flask 100mg of drugs were dissolved in distilled water to give concentrations of 1000 µg/mL and further diluted to obtain suitable concentration for calibration graph.

PROCEDURE FOR THE ASSAY OF PURE DRUG
Into a series of 10ml volumetric flask 1 to 7 aliquots of drugs were transferred. To each flask 1ml of 1 mol/L HCL was added followed by 1ml of NBS solution (70µg/mL). The contents were mixed and the flasks were set aside for 10 min under occasional shaking. Finally 1ml of 50µg/mL Rodamine-B solution was added to each flask. The remaining volume was adjusted with water and the absorbance of the solution was measured at 557 nm against a reagent blank after 10 min. Calibration curves were constructed for all the drugs by plotting the absorbance verses concentrations of the drugs (µg/mL). Calibration curves (figure1) were linear for all the drugs whose limits are mentioned in table (1). Slope intercept, correlation coefficient of the calibration curves are calculated and tabulated.

PROCEDURE FOR TABLETS
1. Olanzapine
For the analysis of pharmaceutical formulations four tablets (Zyprexa, 10mg) were weighed and powdered. A quantity equivalent to 10mg of Olanzapine was transferred into a 100ml volumetric flask and 60ml of 0.1M HCL was added. The contents were shaken thoroughly for about 15 min. The volume was adjusted to the mark with 0.1MHCL mixed well and filtered using Whatman No. 42 filter paper. First 10mL of the portion was rejected and a convenient aliquot of filtrate was diluted with the same diluant to get required concentration for the analysis of the drug.

2. Valacyclovir
Five tablets (Valamac, 500mg) were weighed and powered. The power equivalent to 10mg was dissolved in 0.1N HCL. Then the solution was sonicated for 30 min and filtered. It was further diluted to get required concentration.

3. Dronedarone
To prepare a stock solution for the assay of Dronedarone, ten tablets (Multaq, 400mg) were grounded and the powder equivalent to 10mg of Dronedarone were weighed and
transferred into a 100ml of volumetric flask and dissolved in 25ml of Methanol. The mixture was sonicated for 30 min. Then the contents of the flask were left to return to room temperature and the volume was adjusted with the water. Solution was then filtered through a 0.45µm nylon syringe filter.

4. Donepezil
Twenty tablets (Aricept, 10mg) were accurately weighed, finely pulverized and thoroughly mixed. The powder equivalent to 10mg of Donepezil declared active principle was transferred into 100mL volumetric flask and about 70mL of methanol was added, the contents of the flask were sonicated for 30 min. and then filtered. Aliquots containing suitable concentration of the studied drug was analyzed.

5. Vilazodone
Twenty tablets (Valtrex, 20mg) were crushed to powder and accurately weighed the sample equivalent to 10mg. This powder was transferred into a 100mL volumetric flask. Add about 70mL of diluent and sonicate to dissolve it completely. The flask was made up to the mark with the same solvent and it is further diluted to get required concentration for the analysis of the drug.

RESULTS AND DISCUSSION
The proposed UV spectrophotometric method is indirect and is based on the determination of the surplus NBS after allowing the reaction between drug and measured amount of NBS to be complete. The subsequent determination of the latter by reacting with a fixed amount of Rhodamine-B dye and measuring the absorbance at 557nm. This method is make use of the bleaching action of NBS on the dyes, the decolorisation being caused by the oxidative destruction of the dyes. Drug when added in increasing concentrations to a fixed concentration of NBS, consumes the latter proportionally and their occurs a concomitant fall in the concentration of NBS. When a fixed amount of dye is added to decreasing concentration of NBS, a proportional increase in the concentration of dye leading to a linear increase in the absorbance is observed with increasing concentration of drug. Drug-acid-NBS-dye was the optimum sequence of addition. Other sequences gave lower absorbance value under the same experimental condition.

Preliminary experiments were conducted to determine the maximum concentrations of Rhodamine-B, spectrophotometrically by measuring the absorbance of their acidic solution at their respective λ max and the upper limits were found to be 5µg/mL for Rhodamine-B. NBS concentration of 7µg/ml was found to be sufficient to bleach the red color due to 5µg/mL of Rhodamine-B. Hydrochloric acid was found to be convenient medium for this method. It was found that maximum absorbance was obtained with 1mL of 1 N Hcl, hence a volume of 1mL of 1 N Hcl is used for all measurements. The time required to complete the reaction was found to be 10 min. constant absorbance readings were obtained when the reaction times were extended up to 15 min. And a standing time of 10 min was necessary for the bleaching action of dye by the residual NBS. The measured colour was stable for several hours even in the presence of reaction product.

METHOD VALIDATION
The methods developed have been validated in terms of guidelines of international conference of harmonization (ICH) viz., sensitivity, selectivity, precision, accuracy, linearity, LOD, LOQ sandell’s sensitivity and robustness. The precision is tested by repeating each experiment at least 6 times while the accuracy has been tested by taking known weight of sample and performing recovery experiments. The values %RSD and t- and F tests are in the permissible range of experimental errors. (Table2).

\[
\text{LOD} = 3.3 \frac{s}{S} \\
\text{LOQ} = 10 \frac{s}{S}
\]

Where \(s\) = standard deviation of the intercept (n=6)
\(S\) = slope of Calibration plot

The robustness of the methods were examined by performing the experiments on 3 different spectrophotometers with excellent tally of absorbance values.

The method developed has also been applied for the analysis of pharmaceuticals. The recovery experiments performed show high accuracy and precision and the results are compared with the available validated reported methods on these drugs. The values %RSD and t-and F tests are in the permissible range of experimental errors (Table3). And show that the methods can be used in both pharmaceutical and drug industries.

APPLICATION TO FORMULATIONS
The obtained satisfactory validation results made the proposed procedures suitable for the determination of drugs in tablets. The results obtained by the proposed methods were statistically compared with those obtained by the reported method. Statistical analysis of the
results using t- and F-tests, no significant differences were found between the calculated and theoretical values of both the proposed and the reported methods at the 95% confidence level with respect to accuracy and precision.

CONCLUSION
The proposed method is simple, precise, accurate and economic with good precision and accuracy. With this method one can do analysis simply with cheap chemicals without losing accuracy. This method provides sensitivity comparable to that achieved by sophisticated and expensive techniques like HPLC. Hence this method can be employed as alternatives for routine analysis of bulk sample and tablets.

ACKNOWLEDGEMENT
I am very much thankful to my guide and Head, Department of Chemistry for providing facilities and constant encouragement at every step during the progress of my work.

Table 1: Analytical and statistical parameters of spectrophotometric method

<table>
<thead>
<tr>
<th>Drugs Name Parameters</th>
<th>Olanzapine</th>
<th>Valacyclovir</th>
<th>Dronedarone</th>
<th>Donepezil</th>
<th>Vilazodone</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ max, nm</td>
<td>557</td>
<td>557</td>
<td>557</td>
<td>557</td>
<td>557</td>
</tr>
<tr>
<td>Beer’s law limit (µg/ml)</td>
<td>0.2-1.4</td>
<td>20-140</td>
<td>2.14</td>
<td>5.35</td>
<td>1.7</td>
</tr>
<tr>
<td>Molar absorptivity (L mol⁻¹ cm⁻¹)</td>
<td>321385</td>
<td>1620</td>
<td>25020</td>
<td>7580</td>
<td>52920</td>
</tr>
<tr>
<td>Slope (specific absorptivity)</td>
<td>0.0114</td>
<td>0.0049</td>
<td>0.0321</td>
<td>0.007</td>
<td>0.133</td>
</tr>
<tr>
<td>Intercept, a</td>
<td>-0.002</td>
<td>0.010</td>
<td>0.021</td>
<td>0.0521</td>
<td>0.0079</td>
</tr>
<tr>
<td>Correlation coefficient, r</td>
<td>0.999</td>
<td>0.9986</td>
<td>0.9983</td>
<td>0.9993</td>
<td>0.994</td>
</tr>
<tr>
<td>Sandal’s sensitivity (µg cm⁻¹)</td>
<td>0.0009</td>
<td>0.204</td>
<td>0.0311</td>
<td>0.142</td>
<td>0.0075</td>
</tr>
<tr>
<td>Standard deviation of intercepts (n=6) (Sa)</td>
<td>0.0049</td>
<td>0.0206</td>
<td>0.0366</td>
<td>0.019</td>
<td>0.039</td>
</tr>
<tr>
<td>Standard deviation of slope (Sb)</td>
<td>0.0174</td>
<td>0.02</td>
<td>0.039</td>
<td>0.024</td>
<td>0.031</td>
</tr>
<tr>
<td>Limit of detection (µg/ml)</td>
<td>0.929</td>
<td>0.99</td>
<td>3.046</td>
<td>2.612</td>
<td>4.015</td>
</tr>
<tr>
<td>Limit of quantification (µg/ml)</td>
<td>2.787</td>
<td>2.97</td>
<td>9.138</td>
<td>7.836</td>
<td>12.45</td>
</tr>
<tr>
<td>Regression equation Y=a+bx; x=conc. of drug(µg/ml)</td>
<td>-0.002</td>
<td>0.010</td>
<td>0.021</td>
<td>0.0521</td>
<td>0.0079</td>
</tr>
</tbody>
</table>

Table 2: Determination of accuracy and precision of the methods on pure drug samples

<table>
<thead>
<tr>
<th>Drugs Name Parameters</th>
<th>Olanzapine</th>
<th>Valacyclovir</th>
<th>Dronedarone</th>
<th>Donepezil</th>
<th>Vilazodone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount taken (µg/ml)</td>
<td>0.2</td>
<td>20</td>
<td>2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>0.4</td>
<td>0.4</td>
<td>4</td>
<td>10</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>0.6</td>
<td>0.625</td>
<td>6</td>
<td>15</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Amount found (µg/ml)</td>
<td>0.23</td>
<td>19.9</td>
<td>1.98</td>
<td>5.1</td>
<td>1.01</td>
</tr>
<tr>
<td>0.399</td>
<td>0.397</td>
<td>3.9</td>
<td>10.2</td>
<td>1.99</td>
<td></td>
</tr>
<tr>
<td>0.625</td>
<td>0.601</td>
<td>6.2</td>
<td>15.09</td>
<td>2.98</td>
<td></td>
</tr>
<tr>
<td>% Recovery</td>
<td>99.85</td>
<td>99.15</td>
<td>99.01</td>
<td>100.2</td>
<td>101</td>
</tr>
<tr>
<td>99.71</td>
<td>100.8</td>
<td>99.2</td>
<td>97.5</td>
<td>102</td>
<td>99.5</td>
</tr>
<tr>
<td>% RSD</td>
<td>1.09</td>
<td>0.895</td>
<td>1.292</td>
<td>0.719</td>
<td>1.371</td>
</tr>
<tr>
<td>Proposed mean ± SD</td>
<td>99.78 ± 1.09</td>
<td>99.6 ± 0.895</td>
<td>98.93 ± 1.292</td>
<td>100.9 ± 0.719</td>
<td>99.94 ± 1.371</td>
</tr>
<tr>
<td>Ref Mean ± SD</td>
<td>102.3 ± 1.76 (n=3)</td>
<td>100.31 ± 1.0 (n=3)</td>
<td>100.06 ± 0.259 (n=6)</td>
<td>100.35 ± 0.76 (n=6)</td>
<td>100.01 ± 0.79 (n=6)</td>
</tr>
<tr>
<td>t-test</td>
<td>0.604</td>
<td>0.153</td>
<td>1.923</td>
<td>0.096</td>
<td>0.9</td>
</tr>
<tr>
<td>F-test</td>
<td>3.097</td>
<td>0.801</td>
<td>0.067</td>
<td>0.5776</td>
<td>0.624</td>
</tr>
</tbody>
</table>
### Table 3: Results of assay of tablets by the proposed methods and statistical evaluation and recovery experiments by standard addition method

<table>
<thead>
<tr>
<th>Drugs Name Parameters</th>
<th>Olanzapine</th>
<th>Valacyclovir</th>
<th>Dronedarone</th>
<th>Donepezil</th>
<th>Vilazodone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount taken (µg/ml)</td>
<td>0.350</td>
<td>0.525</td>
<td>0.87</td>
<td>0.7</td>
<td>0.68</td>
</tr>
<tr>
<td>40</td>
<td>60</td>
<td>80</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Amount found (µg/ml)</td>
<td>0.348</td>
<td>0.51</td>
<td>0.68</td>
<td>0.88</td>
<td>0.88</td>
</tr>
<tr>
<td>39.8</td>
<td>60.2</td>
<td>80.4</td>
<td>99.8</td>
<td>99.8</td>
<td>99.8</td>
</tr>
<tr>
<td>% Recovery</td>
<td>99.4</td>
<td>97.5</td>
<td>97.2</td>
<td>100.5</td>
<td>100.5</td>
</tr>
<tr>
<td></td>
<td>99.5</td>
<td>100.3</td>
<td>100.5</td>
<td>99.8</td>
<td>99.8</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.738</td>
<td>0.624</td>
<td>0.349</td>
<td>0.812</td>
<td>1.372</td>
</tr>
<tr>
<td>Proposed mean ± SD</td>
<td>98.55 ± 0.73</td>
<td>100.025 ± 0.62</td>
<td>98.67 ± 0.34</td>
<td>98.58 ± 0.81</td>
<td>99.30 ± 1.37</td>
</tr>
<tr>
<td>Ref Mean ± SD</td>
<td>104.1 ± 1.26</td>
<td>100.61 ± 0.306</td>
<td>100.20 ± 0.2</td>
<td>100.83 ± 0.38</td>
<td>99.64 ± 0.82</td>
</tr>
<tr>
<td>t-test</td>
<td>0.818</td>
<td>1.032</td>
<td>0.914</td>
<td>1.186</td>
<td>2.283</td>
</tr>
<tr>
<td>F-test</td>
<td>1.587</td>
<td>0.093</td>
<td>3.04</td>
<td>0.144</td>
<td>0.0064</td>
</tr>
</tbody>
</table>

**Fig. 1:** Calibration curves of (1) Olanzapine (2) Valacyclovir (3) Dronedarone (4) Donepezil (5) Vilazodone

**STRUCTURE OF DRUGS**

![Olanzapine Structure]
Valacyclovir

Dronedarone

Donepezil

Vilazodone
REFERENCES
19. Sriharia G, Rami Reddya N, Nagarajasetty K and Chakravarthic IE. Spectrophotometric Determination of