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### Colorimetric Approaches To Drug Analysis And Applications – A Review

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#### ABSTRACT

The main purpose of this review is to highlight the importance of colorimetric approaches to drug analysis both in dosage forms as well as biological samples. Colorimetric methods using colorimetric reagents are highly sensitive, specific and an easy way of determining various analytes in a variety of matrices within a short time. The colorimetric procedures discussed are statistically validated and reported in various quality control laboratories. Hence in the present review significance of colorimetric procedures, various reagents used along with principles and applications are mentioned.

**Key words:** Colorimetric approaches, sensitive, matrices, quality control, applications.

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## INTRODUCTION

Colorimetry is a technique which involves the quantitative estimation of colors frequently used in biochemical investigation. Color can be produced by any substance when it binds with color forming chromogens. The difference in color intensity results in difference in the absorption of light. The intensity of color is directly proportional to the concentration of the compound being measured.<sup>1</sup> Wavelength between 380 nm to 780 nm forms the visible band of light in electromagnetic spectrum.

**Table 1: Visible wavelength ranges with colours absorbed and transmitted**

Wavelength (nm)	Colour absorbed	Colour transmitted
380-420	Violet	Green - yellow
420-440	Violet-Blue	Yellow
440-470	Blue	Orange
470-500	Blue-green	Red
500-520	Green	Purple
520-550	Yellow-green	Violet
550-580	Yellow	Violet-blue
580-620	Orange	Blue
620-680	Red	Blue-green
680-780	Purple	Green

The colorimetric procedures or spectroscopic procedures are generally adopted for any of the following reasons:

- The adoption of a visible spectrophotometric procedure instead of an UV procedure, may be based on cost considerations.
- Indirect spectroscopic methods are used to improve the selectivity of the assay of an UV-absorbing substance in a sample that contains other UV-absorbing components.
- If the analyte absorbs weakly in UV region, a more sensitive method of assay is obtained by converting the substance to a derivative with a more intensely absorbing chromophore.
- The interference from irrelevant absorption may be avoided by converting the analyte to a derivative<sup>2</sup>.

### Colorimetry:

Colorimetry or visible spectrophotometry is a technique used to determine the concentration of colored compounds in a solution based on the Beer-Lambert's law which establishes a direct relation between the absorbance and concentration at a given wavelength of maximum absorption. A colorimeter/visible spectrophotometer is a device used to test the concentration of a solution by measuring the absorbance of a specific wavelength of light. Specificity and sensitivity are to be

considered in the selection of a reagent for colorimetric analysis. In spectrophotometric methods, the selectivity depends on nature of the reagent, oxidation state of the metal ion, pH of the medium, temperature, order of mixing reagents, ageing of reagents and the careful assessment of the absorbance properties and stability of the chromophore generated<sup>3</sup>.

Colorimetry has wide applications like study of molar composition of complexes, determination of pKa value of indicators, determination of inorganic complexes and in quantitative analysis. Determination of formula and stability constants of the metal complexes is another important application of visible spectrophotometry in addition to its quantitative analysis. The methods that are commonly used for the optimization of colorimetric procedures and determination of formula of the metal complex are Job's method of continuous variation<sup>4</sup>, mole ratio method<sup>4</sup> and Asmus method.

#### REAGENTS USED IN COLORIMETRIC METHODS:

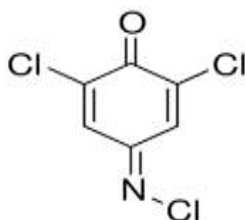
A wide variety of reagents are being used in the development of various colorimetric methods applied for estimations of drugs in varied matrices and in biochemical investigations. Some of them include

1. 2,6-dichloroquinone-4-chloroimide
2. 2,4-Dinitrophenyl hydrazine
3. 2,3,5-triphenyl tetrazolium salt
4. 3-methyl-2-benzothiazolinone hydrazone
5. Bratton-marshall reagent
6. Dimedone
7. Deniges reagent
8. Dyes
9. Folin-coicalteu reagent
10. Froehde reagent
11. Hydroxyl amine
12. Lucas reagent
13. Molybdenum blue
14. Ninhydrin
15. 1,2-naphthoquinone-4-sulphonic sulphonate
16. Para dimethyl amino benzaldehyde
17. Para dimethyl cinnamaledehyde
18. Phospho molybdic acid

## 19. Simon's reagent

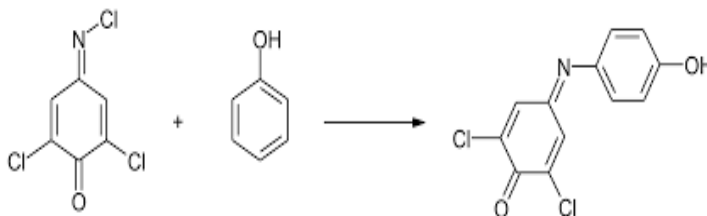
**2,6-dichloroquinone-4-chloroimide:**

Synonym: Gibb's reagent

**Figure 1: Structure of 2,6-dichloroquinone-4-chloroimide**

Principle: When phenolic compounds react with gibb's reagent, coupling reaction may take place.

Phenols: Imide portion of gibb's reagent reacts with phenolic compounds and gives corresponding products coupling with nucleophilic sites by elimination of chlorine.

**Figure 2: Reaction of gibb's reagent with phenols**

Amines: Gibb's reagent couples with amine by elimination of hydrochloric acid and results in coloured complex which is measured at characteristic maximum wavelength.

Preparation of reagent: A solution of 0.5-2% 2,6-dichloroquinone-4-chloroimide in ethanol (reagent stable for 3 weeks when refrigerated).

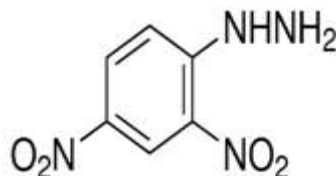
**Applications:**

- Lamotrigine: Drug is mixed with methanol, 1 mL of 0.5% gibb's reagent is added, mixture is heated for 15 minutes (403 nm)<sup>5</sup>.
- Pregabalin: Drug is mixed with methanol, 1.5 mL of 0.5% gibb's reagent is added, mixture is heated for 10 minutes (440 nm)<sup>6</sup>.
- Bisoprolol fumarate: Drug is mixed with isopropanol and 5 mL of gibb's reagent is added and the mixture is heated (532 nm)<sup>7</sup>.
- N-acetyl cysteine: Drug is mixed with absolute ethanol, 1 mL of gibb's reagent and sodium acetate were added, mixed (438 nm).
- Captopril: Drug is mixed with 1 mL dimethyl sulfoxide (DMSO) and 1mL gibb's reagent is added and mixed (443nm)<sup>8</sup>.

- It is used in spectrophotometric determination of phenolic sympathomimetics like Ritodrine Hydrochloride.
- It is a very good reagent for determination of Vitamin B<sub>6</sub>.
- It is used for the identification of un-substituted and p-alkoxy phenols.
- It is used in thin layer chromatography to produce colour spots, for example: Sulphur containing compounds show colored spots when sprayed with Gibbs reagent<sup>9</sup>.

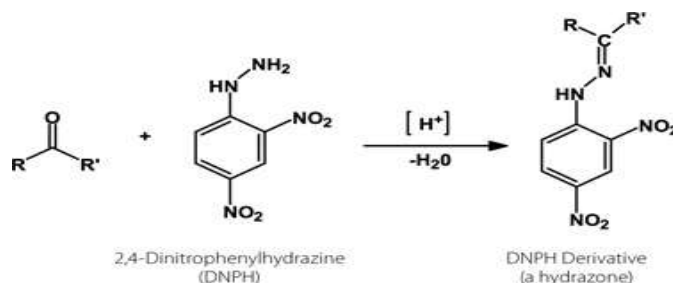
### 2,4-dinitrophenyl hydrazine:

Synonym: Brady's reagent



**Figure 3: Structure of 2,4-dinitrophenyl hydrazine**

Principle: 2, 4-DNP first attaches at the carbon-oxygen double bond to give an intermediate compound which then loses a molecule of water and results in formation of condensed chromogen.



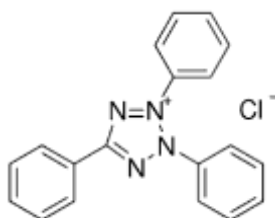
**Figure 4: Reaction of 2,4-DNP with ketone**

### Applications:

- Mainly used to determine aldehydes and ketones.
- Used to determine the drugs spectroscopically like corticosteroids, flavanones, atorvastatin, ezetimibe, valproic acid.

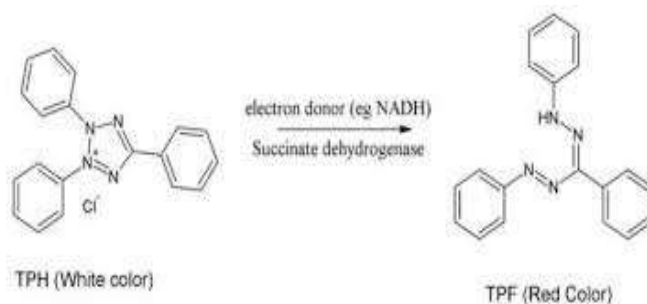
### 2,3,5-triphenyl tetrazolium salt:

Synonym: Tetrazole red



**Figure 5: Structure of 2,3,5-triphenyl tetrazolium salt**

Principle: Triphenyl tetrazolium chloride (TTC) is a redox indicator commonly used in biochemical experiments to indicate cellular respiration. In presence of steroid with a  $\alpha$ -ketol side chain group, tetrazolium salts are reduced to their coloured formazan derivatives. Several formulations containing corticosteroids are assayed using TTC.



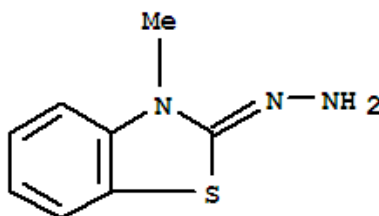
**Figure 6: Reaction of 2,3,5-triphenyl tetrazolium salt**

#### Applications:

- Catecholamines: Epinephrine and norepinephrine react with 2, 3, 5-triphenyl tetrazolium salt in presence of alcohol and 0.1N KOH, gives blue colour (485 nm)<sup>10</sup>.
- Some of the other drugs spectroscopically measured with 2, 3, 5-triphenyl tetrazolium salt are cefepime hydrochloric acid, cefuroxime sodium, isoniazid, and rifampin.

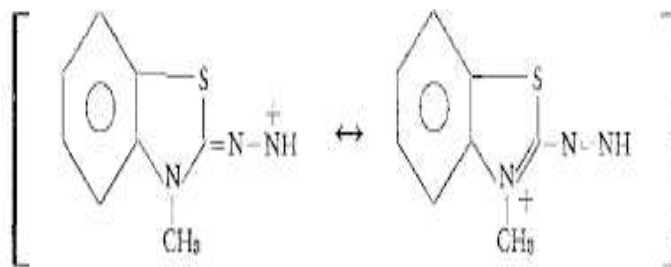
#### 3-methyl-2-benzothiazolinone hydrazone:

Synonym: MBTH, Sawicki's reagent



**Figure 7: Structure of MBTH**

**Principle:** Basic principle is oxidation followed by coupling. MBTH undergoes oxidative coupling reaction catalysed by ferrous ion. Under reaction conditions MBTH loses 2 electrons and one proton forming an electrophilic intermediate which is an active coupling species. This intermediate undergoes electrophilic substitution with the phenols, amines, aldehydes to form coloured product.



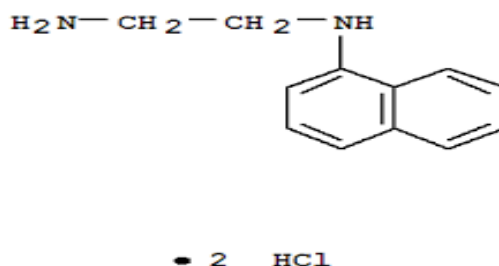
**Figure 8: Electrophilic intermediate of MBTH reagent**

#### Applications:

- Lamotrigine: Drug is dissolved in methanol, to each dilution 2 mL of MBTH and 2 mL FeCl<sub>3</sub> are added, diluted with water which gave blue colour <sup>11</sup>.
- Tenofovir: Drug is mixed with MBTH and this mixture produced apple green in presence of FeCl<sub>3</sub> (626.5 nm)<sup>12</sup>.
- Methyldopa: Drug is mixed with MBTH, potassium ferricyanide and sodium carbonate, pH maintained at 10.4 to form orange water soluble dye (460 nm).
- Nepafenac: Drug is mixed with MBTH solution and potassium permanganate is added which produce blood red chromogen (540 nm)<sup>13</sup>.
- Leukotriene: Drug is mixed with 1.5 mL MBTH and 2 mL FeCl<sub>3</sub>, kept aside and the absorbance of resulting green chromogen measured at 610 nm<sup>14</sup>.

#### Bratton-marshall reagent:

Synonym: Monomethanolate



**Figure 9: Structure of bratton-marshall reagent**

**Principle:** The primary aromatic amino group is first diazotized with sodium nitrite and hydrochloric acid. The excess nitrous acid is neutralized by treating with ammonium sulfamate reagent. Finally, diazonium ion can couple with bratton marshal reagent to produce a highly coloured azodye complex measured at 550 nm.

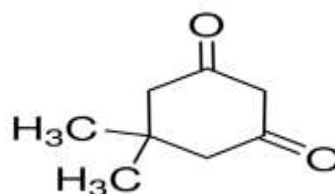
**Preparation:** 100 mg of N-1-naphthyl ethylene diamine chloride dihydrochloride was dissolved in 100 mL of mixture (7 parts of acetone and 3 parts of water).

**Applications:**

- Mesalmine: Drug is mixed with nitrous acid to form diazotized mesalamine to which ammonium sulfamate added followed by coupling with bratton-marshall reagent produces violet chromogen (552 nm)<sup>15</sup>.
- Nimesulide: Drug is mixed with 0.1N hydrochloric acid and zinc dust, heated at 80°C and mixed with sodium nitrite, ammonium sulfamate and bratton marshall reagent, kept aside for 5 minutes (559 nm)<sup>16</sup>.
- Sulfadoxine: Drug is mixed with ice cold sodium nitrite and 1 mL of 2M hydrochloric acid at room temperature. After 5 minutes 1 mL of sulfamic acid, alcoholic diphenyl amine and bratton marshall reagent is added which produces pink chromogen (524 nm)<sup>17</sup>.
- Efavirenz: Drug is mixed with sodium nitrite, 2 mL of 2M hydrochloric acid and bratton marshall reagent, volume made up with water and kept aside produces purple chromogen (523 nm)<sup>18</sup>.
- Ceftazidime: Drug is mixed with 2 mL hydrochloric acid, 1 mL sulfamic acid and 1 mL bratton marshall reagent, left aside for 3 minutes and produces violet chromogen (575 nm)<sup>19</sup>.
- The other drugs estimated are Topiramate (551nm) and Amisulpride (530nm).

**Dimedone:**

Synonym: Cyclomethone



**Figure 10: Structure of dimedone**

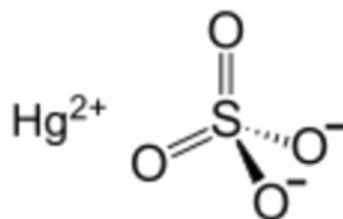
Preparation: Dimedone is prepared from mesityl oxide and diethyl malonate. Applications: Dimedone is used for the colorimetric determination of paracetamol and oxyphenbutazone both in pure form and in their tablets. Dimedone can be used in the industry of organic synthesis.

**Denige's reagent:**

Synonym: Mercury (II) sulphate

Structure:

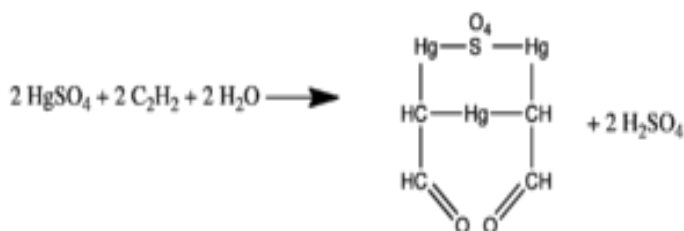




**Figure 11: Structure of denige's reagent**

Preparation: Prepared by dissolving 5 grams of HgO in 20 mL of concentrated sulfuric acid and 100 mL of distilled water.

Principle: The main principle is dehydration. This reagent is used to detect tertiary amines which can be easily dehydrated to form isoolefin in the presence of acid.



**Figure 12: Reaction of denige's reagent**

### Dyes:

Acid-dye method: The addition of amine in its ionized form to an ionized acidic dye like methyl orange yields a salt that may be extracted into an organic solvent such as chloroform. The indicator dye is added in excess and the  $p^H$  of the aqueous solution is adjusted if necessary to a value where both the amine and dye are in the ionized forms. The ion-pair is separated from the excess indicator by extraction into the organic solvent and absorbance is measured at the maximum wavelength of the indicator in the solvent.<sup>2</sup>

### Applications:

- The acid-dye technique is used for the assay of formulations containing certain quaternary ammonium salts or amines like biperidine lactate injection, clonidine hydrochloride injection and tablets.
- Sibutramine hydrochloride: Drug is added with 5 mL of dye; total volume was made up to 20mL with water, to this 10 mL of chloroform was added and the contents were shaken for 5 min. The organic layer was separated as it produces yellow colored solution (415 nm)<sup>20</sup>.
- Telmisartan: Metanil yellow dye was used (420 nm)<sup>21</sup>.

- Amantadine: Bromocresol green (415 nm), bromophenol blue (412 nm) and bromothymol blue (414 nm) were used for determination of anti-parkinsonian drug in formulations and biological samples<sup>22</sup>.

### **Folin-coicalteu reagent:**

Synonym: Folin's phenol reagent, Folin-Denis reagent

It is a mixture of phosphomolybdate and phosphotungstate and is used for invitro assay of phenolic and polyphenolic antioxidants, also reacts with thiols, many vitamins, nucleotide base guanidine, triose glyceraldehyde and dihydroxyacetone and some inorganic ions. The composition is as follows:



Principle: The main principle is reduction. When FC reagent reacts with the drug in presence of reducing agents like stannous chloride ( $\text{SnCl}_2$ ) and ascorbic acid, probably drugs effect reduction of one or more oxygen atoms from tungstate or molybdate in the FC reagent, there by producing one or more possible reduced species which have characteristic intense blue colour.

Preparation: Dissolve 10 gm sodium tungstate and 2.5 gm sodium molybdate in 70 mL water, add 5 mL of 85% phosphoric acid and 10 mL concentrated hydrochloric acid and reflux for 10 hours. To this mixture add 15 g lithium sulphate, 5 mL water and 1 drop bromine and again reflux for 15 minutes. Now cool it to room temperature and make up the volume to 100 mL with water.

### **Applications:**

- Doripenum: Drug is mixed with 2 mL sodium carbonate and FC reagent, volume made up with distilled water and kept aside which produces blue colour (725 nm)<sup>23</sup>.
- Ertapenem: Drug is mixed with sodium carbonate and FC reagent and the blue colour produced is measured at 848 nm<sup>23</sup>.
- Flucloxacillin: Drug is mixed with 2 mL NaOH and 0.5mL FC reagent which produces pale blue colour (912 nm)<sup>24</sup>.
- Potassium: Estimation of potassium from serum is carried out by precipitating it as cobalt nitrite and subsequent estimation of one of the constituents of the precipitate is done using FC reagent<sup>25</sup>.
- Citalopram hydrobromide: Drug is mixed with 2 mL NaOH and 2 mL FC reagent and kept aside for 15 minutes which produces pale blue colour (730 nm)<sup>26</sup>.

- The other drugs estimated using FC reagent are aspirin, diazepam, ampicillin, procaine hydrochloric acid, doxycycline, tannins and phenols.

### Froehde reagent:

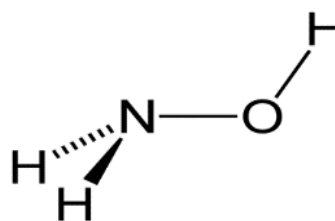
It is a simple spot test to presumptively identify alkaloids, especially opioids. It is composed of a mixture of molybdic acid or a molybdate salt dissolved in hot, concentrated sulfuric acid, which is then dripped onto the substance being tested.

### Applications:

- Methcathinone: Drug is mixed with 5% w/v sodium molybdate in hot sulphuric acid and 2-3 drops reagent gives yellow colour<sup>27</sup>.
- Amphetamine-type stimulants (ATS): Different ATS drugs like amphetamine, d-methamphetamine, phentermine, etc., were tested with this reagent and produced a variety of colors like pale yellow, light orange and light brown<sup>28</sup>.
- Phenylalkylamine: Drug is mixed with 0.5 g molybdic acid (or) sodium molybdate in 100 mL of hot concentrated sulphuric acid and few drops of reagent gives yellow or green colour.
- Morphine: Drug is mixed with sodium molybdate in hot sulphuric acid and few drops of reagent kept aside produces red colour<sup>29</sup>.
- Allylescaline: Drug is mixed with 0.5 g molybdic acid, 100 mL of concentrated 95-98% sulphuric acid, 2-3 drops of reagent and kept aside which produces green or black colour.
- The other drugs estimated using froehde reagent are tetracycline and promethazine hydrochloric acid.

### Hydroxyl amine:

Synonym: Hydroxylazane



**Figure 13: Structure of hydroxyl amine**

Principle: The basic principle is oxidation. The hydroxyl amine reacts with aldehydes and ketones to give oximes. The nucleophilicity of the nitrogen on the hydroxyl amine is increased by the presence of oxygen. Successive proton transfers allow for elimination of water.



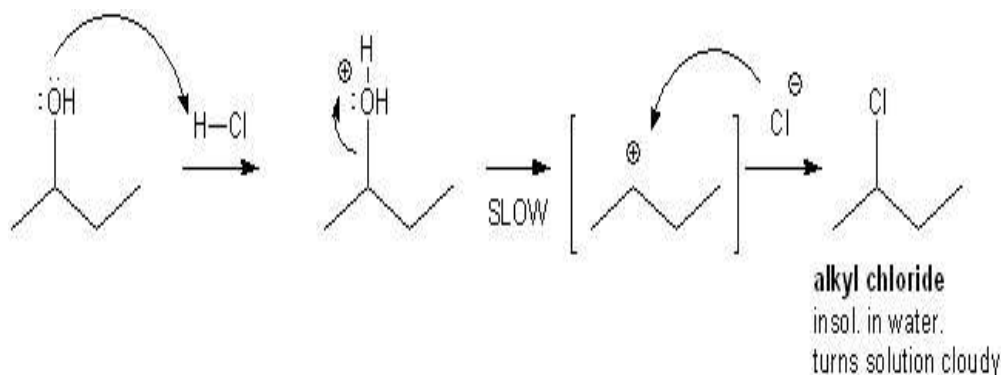
**Applications:**

- Penicillin in broth has been colorimetrically estimated using hydroxylamine<sup>30</sup>.
- Volatile esters have been estimated with hydroxylamine in alkaline solution to form hydroxamic acid which in turn reacts with ferric ion to form a red ferric hydroxamate complex<sup>31</sup>.

**Lucas reagent:**

Lucas' reagent is a solution of anhydrous zinc chloride in concentrated hydrochloric acid. This solution is used to differentiate between primary, secondary, and tertiary alcohols based on the turbidity.

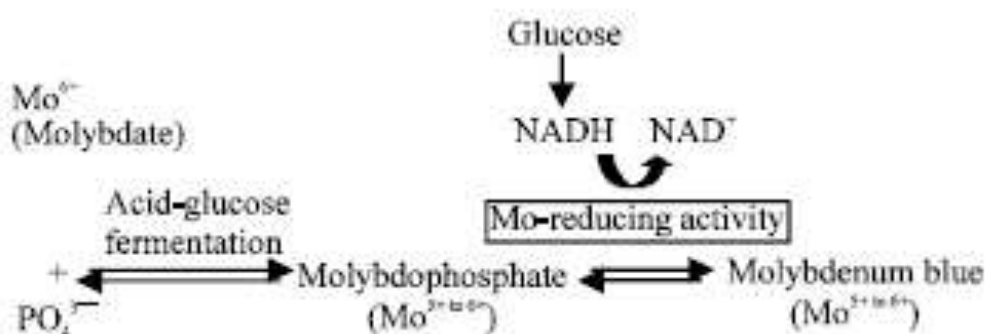
Principle: The basic reaction is substitution reaction in which chloride replaces hydroxyl group. It is based on the difference in reactivity of primary, secondary, tertiary alcohols in which primary carbocations are less stable and tertiary carbocations are stable.



**Figure 14: Reaction of lucas reagent**

**Molybdenum blue:**

Principle: The basic principle involved is reduction. The colour arises because the near colourless anion can accept more electrons to form an intensely coloured mixed valence complex. Reduction process is reversible.



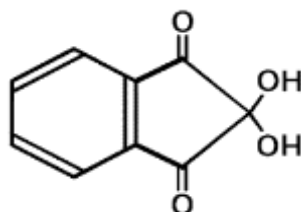
**Figure 15: Reaction of molybdenum blue**

**Applications:**

- Ascorbic acid: In acidic medium phosphates bond with ammonium molybdate to form ammonium phosphomolybdate with reducing agent (ascorbic acid). Ammonium phosphomolybdate reduced to form a bluish-purple coloured complex (882 nm)<sup>32</sup>.
- Tartrazine: Tartrazine is first mixed with industrial grade NaCl and later molybdenum blue is added which produces purple bluish colour (560 nm).
- Pyrogallol: Drug is mixed with molybdenum blue and pyrogallol molybdo albumin solution and pH maintained at 2.5 (600 nm)<sup>33</sup>.
- Nicorandil: Drug is mixed with phosphomolybdenum blue and vanadium chloride, volume made up with distilled water and decrease in absorbance measured at (827 nm)<sup>34</sup>.
- Nifedipine: Drug mixed with KOH in DMSO, molybdenum blue mixed and kept aside (430 nm, method A). Oxidation of drug with ammonium molybdate and subsequently reduced molybdenum blue with citric acid (830 nm, method B)<sup>35</sup>.
- The other drugs estimated using molybdenum blue are paracetamol at 830nm, carbamazepine at 823nm, oxycarbamazepine at 824.5nm.

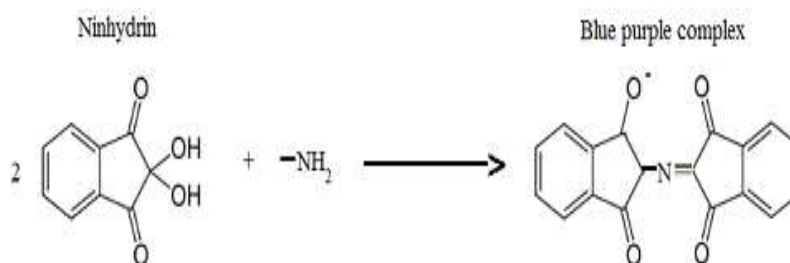
**Ninhydrin:**

Synonym: 2, 2-dihydroxyindane-1, 3-dione



**Figure 16: Structure of ninhydrin reagent**

Principle: Used to detect ammonia, primary and secondary amines. When reacting with these free amines a deep blue or purple colour known as Rhuemann's purple is produced.



**Figure 17: reaction of ninhydrin**

Preparation: 4g of ninhydrin is dissolved in 100mL of 2-methoxy ethanol and shaken with 1g of cation exchanger resin and filtered. To the filtrate 100mL 0.16% w/v of stannous chloride in acetate buffer is added.

### Applications:

- Cyanide: 0.5 mL of ninhydrin reagent is added to cyanide and potassium hydroxide solution that gave deep-red (485 nm) or deep-blue color (590 nm)<sup>36</sup>.
- Metformin hydrochloride: To the standard solution of metformin hydrochloride, 1.5 mL of 5M NaOH, 2.2 mL of 1% ninhydrin solution and 10 mL of water is added, heated on a water bath for 30 min, cooled and volume adjusted to 25 mL with water (572 nm)<sup>37</sup>.
- The other drugs estimated using ninhydrin reagent are cefadroxil, levodopa, gentamicin, hexamine.
- Primarily used to detect ammonia by giving deep blue colour.
- Growth of peptide chain is maintained by ninhydrin.
- Amine, carbamates and amides can be detected by ninhydrin in TLC.

### 1, 2-naphthoquinone-4-sulphonic sulphonate:

Synonym: NQS reagent

Principle: NQS treated with any amine containing compound that will release the hydroxyl group and the sodium sulphonate group is replaced with the aromatic amine group.

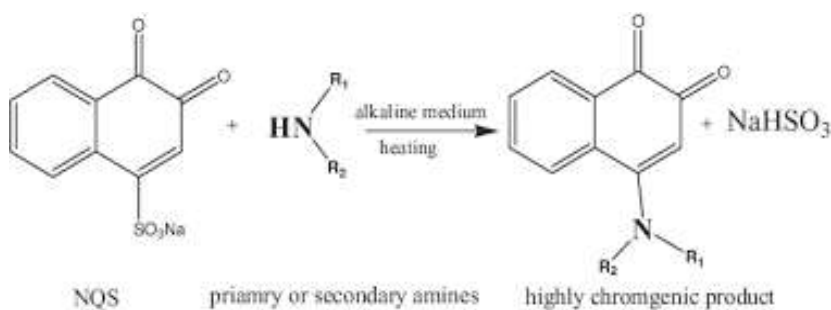


Figure 18: Reaction of NQS reagent

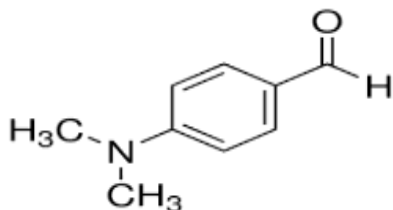
### Applications:

- Finasteride: Drug is transferred into 10 mL volumetric flask, 1.0 mL of 0.3% sodium-1,2-naphthoquinone-4-sulfonate is added followed by 1.0 mL pH 13.0 Na<sub>2</sub>HPO<sub>4</sub> buffer solution and volume made up with distilled water (447 nm)<sup>38</sup>.
- Imipenem: Standard solutions (0.5 to 3.0 mL) of imipenem were transferred to each flask, 2.0 mL of NaOH, 1.5mL of 0.5% NQS in water were successively added and kept aside for 5 minutes. The solutions were made up to volume with distilled water (449 nm)<sup>39</sup>.

- Bicalutamide: Standard drug solutions were transferred into series of 10 mL graduated test tubes, 0.5 mL of NQS and 2 mL of NaOH were added to each test tube, mixed well and volume was made up to 10 mL with methanol (453 nm)<sup>40</sup>.
- Primaquine: Drug is transferred to 10 mL volumetric flask, 2.0 mL of NQS is added followed by 1.5 mL of p<sup>H</sup> 10 buffer solution, the flask is completed to volume with distilled water (485 nm)<sup>41</sup>.
- Cinacalcet hydrochloride: To 1mL of cinacalcet solution, 0.5 mL of tris buffer solution of pH 8.5 and 1 mL of NQS solution were added, allowed to stand at room temperature (25 ± 2°C) for 10 minutes and diluted to volume with water to produce an orange-red colored product (490 nm)<sup>42</sup>.
- The other drugs estimated using NQS reagent are paroxetine, cephalosporins, linagliptin, ramipril, modafinil.
- NQS was used to determine drugs which contain amines and amino acids. This reagent is used in determination of aromatic amines (amphetamine) in industrial waste.

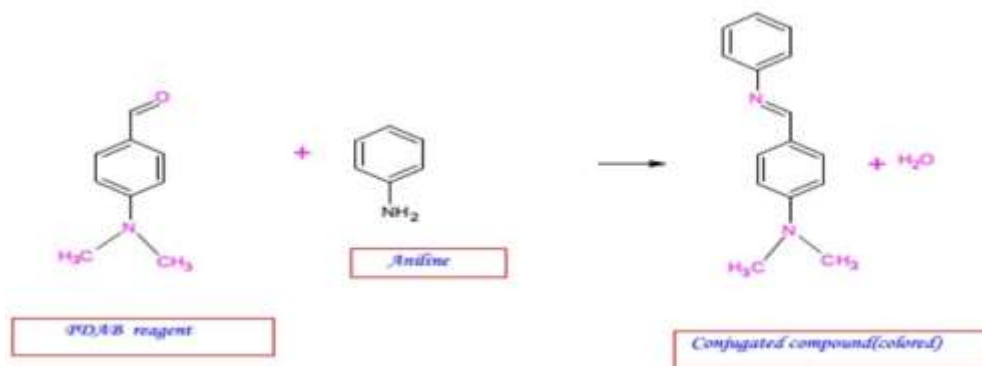
#### Para dimethyl amino benzaldehyde:

Synonym: PDAB, Ehrlich's reagent.



**Figure 19: Structure of PDAB**

Principle: The principle involved in PDAB reagent is formation of condensation products referred as schiff's base. The primary amine group which is present in the structure of the drug reacts with carbonyl group in the PDAB reagent and forms schiff's base.



**Figure 20: Reaction of PDAB**

Preparation: 400 mg of PDAB was mixed with 2 mL of concentrated H<sub>2</sub>SO<sub>4</sub> and 10mL alcohol and finally volume made up to 100mL with distilled water.

### Applications:

- Metronidazole: Drug is mixed with zinc powder and 5N Hydrochloric acid at room temperature in methanol and the resulting amine was then subjected to condensation reaction with aromatic aldehyde PDAB to yield yellow coloured schiff's base (494 nm)<sup>43</sup>.
- Ranitidine hydrochloride: Drug is mixed with PDAB reagent and a colored condensation product was measured at 503 nm<sup>44</sup>.
- Ambroxol: Drug is mixed with 2 mL concentrated hydrochloric acid, 2 mL of 5% PDAB, heated on water bath for 10 minutes and the yellow coloured Schiff's base produced in the flasks were cooled and solutions made up with water (423 nm)<sup>45</sup>.
- Chloramphenicol: Drug mixed with PDAB, concentrated hydrochloric acid and zinc dust gives yellow coloured schiff's base (436.5 nm)<sup>46</sup>.
- Alfuzoin: Drug is dissolved in methanol, 1 mL PDAB reagent and 3mL of sulphuric acid are added, heated for 25 minutes, solutions are cooled, and the volume made up with methanol (565 nm)<sup>47</sup>.

### Para dimethyl amino cinnamaldehyde:

Synonym: PDAC reagent.

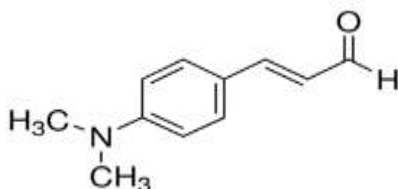


Figure 21: Structure of PDAC

Principle: The the reaction of PDAC reagent with aromatic amines produces schiff's base (condensation). Certain amines condense with various aldehydes in strongly acidic media to give products that are oxidizable to give colour.

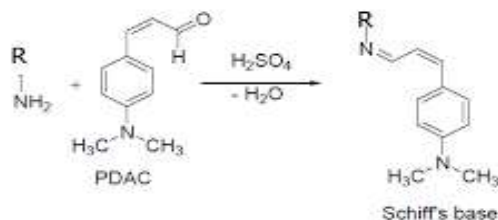


Figure 22: Reaction of PDAC reagent

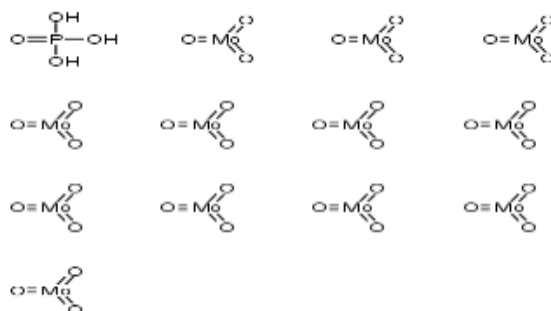


**Applications:**

- Aceclofenac: Drug is mixed with 3 mL of 0.25% w/v PDAC, 1mL per chloric acid, mixture heated at 90° C for 10 minutes with occasional shaking, cooled and volume made up with methanol (658 nm)<sup>48</sup>.
- Alfuzoin: Drug is dissolved in methanol, 1 mL PDAC and 3 mL sulphuric acid is added, mixture is heated for 25 minutes and the volume made up with methanol (560 nm)<sup>49</sup>.
- Zolmitriptan: Drug is mixed with 1 mL PDAC and 1 mL of concentrated sulphuric acid, volume made up with methanol to produce a red chromogen (560 nm)<sup>50</sup>.
- Mesalamine: Drug is mixed with PDAC reagent, 2 minutes later volume made up with water to produce a reddish brown coloured product (524nm)<sup>51</sup>.
- Oseltamivir: Drug mixed with 2mL PDAC and 0.5 mL sulphuric acid and volume adjusted with methanol to give orange red colour (530nm)<sup>52</sup>.

**Phospho molybdic acid:**

Synonym: Dodecamolybdophosphoric acid, PMA



**Figure 23: Structure of phospho molybdic acid**

Principle: It is widely employed as a reagent in the quantitative analysis of several drugs. It forms insoluble adduct (yellowish precipitates) with various groups of drugs and released PMA from the adduct is usually measured by colorimetry due to blue-green colour formation by the reduction of PMA. Reductants like ascorbic acid or hydrazine hydrate can be used to yield molybdenum blue.

**Applications:**

- Trazodone: Drug is mixed with 3 mL 0.01N hydrochloric acid and 1.5 mL PMA, centrifuged, precipitate is collected and acetone is added to dissolve the precipitate. 1 mL each of cobalt nitrate and EDTA are added and heated to 60°C and volume made up with distilled water (840 nm)<sup>53</sup>.

- Gemifloxacin: A spectrophotometric method based on oxidation reaction between the drug and PMA was developed for the determination of gemifloxacin in dosage form and human urine (794 nm)<sup>54</sup>.
- Cabergoline: Drug is mixed with 3 mL water and 2 mL PMA reagent, centrifuged, the precipitate is collected and 5 mL acetone is added to dissolve the precipitate. 1 mL cobalt nitrate and EDTA are added to reduce the released PMA to molybdenum blue and heated to 60°C (840 nm)<sup>55</sup>.
- Flupenthixol: Drug is mixed with 0.25 mL of conc. hydrochloric acid, volume adjusted to 3 mL with water, 1.5 mL phosphomolybdic acid is added, centrifuged, precipitate dissolved in 5 mL of acetone, transferred to 25 mL graduated test tubes, 1 mL each of cobalt nitrate and EDTA are added, heated at 60-70°C for 10 min. and measured at 840 nm<sup>56</sup>.

#### **Simon's reagent<sup>57</sup>:**

The reagent is a mixture of sodium nitroprusside, sodium carbonate and acetaldehyde and is a relatively selective reagent for aromatic secondary amines. A positive reaction will be reflected as a light or dark blue colour, at lower concentration of test compound often after several minutes. It is a secondary amine reacting with acetaldehyde to form an enamine, which is subsequently reacted with sodium nitroprusside to give an immonium salt which is subsequently hydrolysed to simon-awe complex. It is characterized by its blue colour.

Preparation: Solution A: Dissolve 1 g of sodium nitroprusside in 50 mL of distilled water and add 2 mL of acetaldehyde to the solution with thorough mixing.

Solution B: 2 percent sodium carbonate in distilled water.

Add 1 volume of solution A to the drug, followed by 2 volumes of solution B.

#### **Applications:**

- Amphetamine: Drug mixed with simon's reagent produces blue colour.
- Ephedrine: Drug mixed with simon's reagent produces light blue colour.
- Norepedrine: Drug mixed with simon's reagent produces olive green colour.
- N-Methyl ephedrine: Drug mixed with simon's reagent produces light pink colour.
- Cathinone: Drug mixed with simon's reagent produces light brown colour.

#### **CONCLUSION:**

In physical and analytical chemistry, colorimetric assays using reagents that undergo a measurable colour change in the presence of the analyte are widely used. In biochemistry, they are used to test for the presence of enzymes, specific compounds, antibodies, hormones and many more analytes.

Many pharmaceutical substances can be conveniently determined in dosage forms and biological matrices by using different colorimetric reagents with the help of spectrophotometric and chromatographic methods. Further the colorimetric methods offer great sensitivity when compared with UV spectroscopic methods. An additional advantage of the visible spectrophotometric methods is that the absorbance is measured at longer wavelengths where the interference from excipients is less. Most of the procedures are inexpensive and reagents easily available. There is a large scope for development of new reagents and hence colorimetric methods which can be employed for the routine analysis of drugs in a wide variety of matrices.

#### REFERENCES:

1. Michael LB, Edward PF, Larry S. A book of medical science. 5<sup>th</sup> ed., Wolters Kluwer; 2004.
2. Beckett AH, Stenlake JB, Practical pharmaceutical chemistry. 4<sup>th</sup> ed., Part two, New Delhi: CBS Publishers; 2006: 300-301.
3. Olajire AA. Chemical derivatization methodologies for UV-Visible spectrophotometric determination of pharmaceuticals. *International Journal of Pharmaceutical Sciences Review and Research* 2012; 14: 6-24.
4. Stanley B, Jacob AM. Job's Method of Continuous Variations with Ion Exchange for the Study of Complexes in Solution. *J. Phys. Chem.* 1964; 68: 258–260.
5. Chandan RS, Vasudevan M, Deecaraman, Gurupadayya BM, Indupriya M. Spectrophotometric determination of lamotrigine using Gibb's and MBTH reagent in pharmaceutical dosage form. *JPR solutions* 2011; 1813-1815.
6. Sowjanya K, Thejaswini JC, Gurupadayya BM, Indupriya M. Spectrophotometric determination of pregabalin using gibb's and MBTH reagent in pharmaceutical dosage form. *Der Pharma Chemica* 2011; 3: 114.
7. Bhusnure OG, Dongare RB, Gholve SB, Rajmane T, Munde AB, Giram PS. Development and validation of UV spectroscopic method for the determination of bisoprolol fumarate tablets. *International Journal of Pharmacy and biological Sciences* 2018; 334-344.
8. Nahed EE, Fathalla B, Rizk MS. Novel spectrophotometric sethod for the assay of captopril in dosage forms using 2,6-Dichloroquinone-4-Chlorimide. *International Journal of Biomedical science* 2016; 147-152.
9. Tharun. Gibbs reagent. *Saudi Pharmaceutical Journal* 2009; 289-293.

10. Rabbat NA, Omar NM. Colorimetric determination of catecholamines by 2,3,5-triphenyltetrazolium chloride. *Journal of Pharmaceutical Sciences* 1978; 779-81.
11. Chandan RS, Vasudevan M, Deecaraman, Gurupadayya BM, Indupriya M. Spectrophotometric determination of lamotrigine using Gibb's and MBTH reagent in pharmaceutical dosage form. *JPR solutions* 2011; 1813-1815.
12. Mannem SV, Raghavendra B, Yenumula P, Ravi K. Development of new spectrophotometric method for estimation of tenofovir disoproxil fumarate using MBTH reagent. *International Current Pharmaceutical Journal* 2015; 4: 378-381.
13. Sadhana R, Chhaya S, Madhuri B. Development and validation of UV and RP-HPLC method for estimation of nepafenac in bulk drug and ophthalmic formulation. *Journal of Advanced Pharmacy Education & Research* 2015; 15-19.
14. Shanmukha JV, Ramachandran D, Settaluri VS, Shechinah Felice C. Spectrophotometric methods for estimation of leukotriene receptor antagonist in bulk dosage forms. *Rasayan Journal of Chemistry* 2010; 166-171.
15. Patel KM, Patel CN, Panigrahi B, Parikh AS, Patel HN. Development and validation of spectrophotometric methods for the estimation of mesalamine in tablet dosage forms. *Journal of Young Pharmacists* 2010; 2: 284-288.
16. Rajan JP, Shruti SS, Chandani DP, Rajnikant BM, Suhagia BN. Analysis of nimesulide in bulk and tablet formulation by colorimetric method using bratton marshall reagent. *JCPS* 2014; 152-158.
17. Sangita S, Madhurjya N, Vipul P, Hiren P, Dipti D. Spectrophotometric estimation of sulfadoxine in pharmaceutical preparations. *E-Journal of Chemistry* 2010; 7: 1246-1253.
18. Sunitha PG. Development and validation of spectrophotometric methods for the determination of efavirenz in tablets. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 2013; 229-232.
19. Nguyen TD, Doan TD, Giap DH, Nguyen AS. Development of spectrophotometric method for determination of ceftazidime with the bratton marshall reagent in pharmaceutical preparation. *Journal of Science and Technology* 2017; 220-228.
20. Chennaiah M, Veeraiah T, Vinod TK. Extractive spectrophotometric methods for determination of sibutramine hydrochloride using acidic triphenylmethane dye. *International Journal of Chemical Sciences* 2011; 9: 1451-1462.

21. Akram MED, Sameh MH, Ahmed AS. Application of bromocresol green and bromothymol blue for the extractive spectrophotometric determination of anti-hypertensive drugs. *Journal of Applied Pharmaceutical Science* 2015; 5: 122-129.
22. Hany AO, Alaa SA. Extractive-spectrophotometric methods for determination of anti-parkinsonian drug in pharmaceutical formulations and in biological samples using sulphonphthalein acid dyes. *Journal of Saudi Chemical Society* 2012; 16: 75-8.
23. Raghu KB, Aruna K, Vijaya KL. Spectrophotometric determination of doripenem, ertapenem in bulk and injection formulations by FC reagent. *International Journal of Pharmaceutical Sciences and Drug Research* 2013; 5: 184-186.
24. Vijaya RL, Kiranmai K, Dhanaraju MD. Spectrophotometric determination of flucloxacillin sodium using folin-ciocalteu reagent. *Oriental Journal of Chemistry* 2010; 26: 1135-1138.
25. Abul-Fadl MAM. Colorimetric determination of potassium by folin-ciocalteu phenol reagent. *Biochemical Journal*. 1949; 44: 282–285.
26. Asad R, Tariq MA. Spectrophotometric determination of citalopram in pharmaceuticals using folin-ciocalteu reagent. *Asian Journal of Chemistry* 2013; 25: 8913-8916.
27. Kaitlyn ET, Shanlin F, Ronald GS, Nadine K. Color tests for the preliminary identification of methcathinone and analogues of methcathinone. *Microgram Journal* 2012; 9: 27-32.
28. Tang YP, Lim SC, LimSH, Linda BLL. Presumptive testing of amphetamine-type stimulants via colour tests. *ASEAN Journal on Science and Technology for Development* 2010; 27: 66-75.
29. Balls AK, William AW. The determination of morphine. *Journal of Biological Chemistry* 1928; 80: 379-402.
30. Makoto ODA, Minoru H. Colorimetric estimation of penicillin by hydroxylamine method (1). *Nippon Nōgeikagaku Kaishi* (Published by Japan Society for Bioscience, Biotechnology, and Agrochemistry) 1950; 23: 237-239.
31. Adriene RT. A colorimetric method for the determination of esters. *Australian Journal of Scientific Research, Series A: Physical Sciences* 1950; 3: 128.
32. Zhongqi H, Wayne CH. A modified molybdenum blue method for orthophosphate determination suitable for investigating enzymatic hydrolysis of organic phosphates. *Communications in Soil Science and Plant Analysis* 2005; 36: 1373–1383.
33. Yoshikazu F, Itsuo M, Shoko K. Color reaction between pyrogallol red – molybdenum complex and protein. *The Japan Society for Analytical Chemistry*, 1983; 32: E379-E386.

34. El-Adl, El-Sadek, Saeed. Spectrophotometric determination of nicorandil in bulk and pharmaceutical formulation using phospho-molybdenum blue complex. *Journal of Applied Pharmacy* 2017; 9: 3.
35. Rahman N, Azmi SN. New spectrophotometric methods for the determination of nifedipine in pharmaceutical formulations. *Acta Biochimica Polonica* 2005; 52: 915-22.
36. Ioan-marius mihaescu. Cyanide determination with a modified ninhydrin reagent using n-acetyl cysteine in the presence of heavy metal ions. *Journal of the Chilean Chemical Society* 2010; 55.
37. Mubeen G, Khalikha N. Spectrophotometric method for analysis of metformin hydrochloride. *Indian Journal of Pharmaceutical Sciences* 2009; 71: 100–102.
38. Sally MA, Abdalla AE. Development and validation of spectrophotometric method for determination of finasteride in pharmaceutical formulation using 1,2-naphthoquinone-4-sulfonate (NQS). *Journal of Analytical and Bioanalytical Techniques* 2015; 6: 247.
39. Raghu KB, Aruna NK, Vasundhara A, Vijayalakshmi R. Spectrophotometric determination of imipenem in bulk and injection formulations by 1,2-naphtho quinone 4-sulphonic acid (NQS) reagent. *American Journal of Phytomedicine and Clinical Therapeutics* 2014; 2: 126-133.
40. Murali VB, Venkateswara JR, Mushraff AK, Sharma VC, Sreedevi K. Visible spectrophotometric determination of bicalutamide in bulk drug and pharmaceutical formulations. *International Journal of Chemical Science* 2009; 7: 1606-1612.
41. Aida Makram NA, Abdalla AE. Spectrophotometric method for determination of primaquine in pharmaceutical formulations via derivatization with 1,2-naphthoquinone-4-sulfonate. *Austin Journal of Analytical and Pharmaceutical Chemistry* 2014; 1: 1-4.
42. Ibrahim AD, Mona MA, Manal AE. Novel spectrophotometric method for determination of cinacalcet hydrochloride in its tablets via derivatization with 1,2-naphthoquinone-4-sulphonate. *Chemistry Central Journal* 2012; 6: 11.
43. Siddapa K, Mallikarjun M, Reddy PT, Tambe M. Spectrophotometric determination of metronidazole through schiff's base system using vanillin and PDAB reagents in pharmaceutical preparations. *Ecletica Química* 2008; 33.
44. Narayana B, Krishnamurthy A, Divya NS, Kunnummel V. Spectrophotometric determination of ranitidine hydrochloride based on the reaction with p-dimethylaminobenzaldehyde. *Eurasian Journal of Analytical Chemistry* 2010; 5: 63–72.

45. Siddappa K, Prashant CH. Spectrophotometric quantitative determination of ambroxol hydrochloride in bulk and pharmaceutical dosage forms using PDAB reagent. *International Journal of Pharmaceutical Sciences and Research* 2014; 5: 4188-4194.
46. Abbas NA, Dheyaa YA. New spectrophotometric method for the determination of chloramphenicol in pharmaceutical preparations based on schiff base reaction with p-dimethylamino benzaldehyde as reagent. *International J ChemTech Rese* 2016; 9: 712-722.
47. Gowri DS, Vamsi KM, Pawar AKM. Application of condensation reactions for the estimation of alfuzosin hydrochloride in bulk drugs and in pharmaceutical formulations. *Analytical Chemistry: An Indian Journal* 2007; 3: 204-207.
48. Bose A, Dash PP, Sahoo MK. Simple spectrophotometric methods for estimation of aceclofenac from bulk and formulations. *Pharm Methods* 2010; 1: 57-60.
49. Gowri SD, Vamsi KM, Pawar AKM. Application of condensation reactions for the estimation of Alfuzosin hydrochloride in bulk drugs and in pharmaceutical formulations. *Analytical Chemistry an Indian Journal* 2007; 3: 204-207.
50. Saifulla PK, Raveendra PR, Krishna VR. Visible spectrophotometric methods for the determination of zolmitriptan in bulk and pharmaceutical formulations using aromatic aldehydes and folin's reagent. *International J ChemTech Research* 2013; 5: 2941-2946.
51. Navya SS, Gurupadayya BM, Aswani CK. Spectrophotometric determination of mesalamine by PDAC and NQS reagents in bulk and tablet dosage form. *Journal of Pharmacy Research* 2011; 4: 39-41.
52. Kiran KK, Appalaraju N. Spectrophotometric estimation of oseltamivir in pharmaceutical formulations. *Asian Journal of Chemistry* 2009; 21: 5984-5988.
53. Prasad AVVS, Sastry CSP. Cobalt (II)-EDTA complex as a new reductant for phosphomolybdic acid used for the assay of trazodone. *Proc. Indian Acad. Sci. (Chem. Sci.)* 2003; 115: 29-32.
54. Dalia Z, Omnia AI, Wafaa SH, Abdalla S. Simple spectrophotometric and conductometric methods for determination of gemifloxacin in pure, pharmaceutical dosage form and human urine. *Journal of Applied Pharmaceutical Sciences* 2016; 6: 136-143.
55. Rambabu C, Jyothirmayee CA, Nagaraju K. Spectrophotometric determination of cabergoline in tablet dosage forms. *International Journal of Pharmaceutical, Chemical and Biological Sciences* 2012; 2: 72-77.
56. Jameelunnisa B, Abdul R. Estimation of flupentixol in bulk and its pharmaceutical formulations by precipitation reagents. *Asian Journal of Chemistry* 2008; 20: 4163-4167.

57. Karl-Artur K, Martina L. Chemistry and reaction mechanisms of rapid tests for drugs of abuse and precursors chemicals. United Nations Scientific and Technical Notes, Federal Republic of Germany; 1989.

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