Emerging Trends In Colorimetric Approaches For Drug Analysis: An In-Depth Review of Methods And Applications

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Abstract- *This review aims to show how important colorimetric methods are for drug analysis in both medicines and biological samples. Colorimetric methods use special reagents to quickly and accurately detect different substances. These methods are well-tested and widely used in quality control labs. The review explains why colorimetric techniques are important, the different reagents used, and how these methods work and are applied.*

Keywords- Colorimetric approaches, sensitive, matrices, quality control, applications, color, samples, molecules.

I. 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.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**

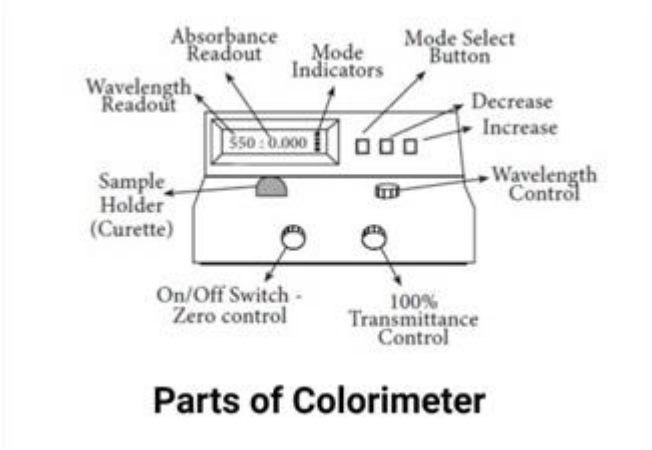
Wavelength (nm)	Colour absorbed	Colour transmitted
380 – 420	Violet	Green- yellow
420-440	Violet – blue	Yellow
440-470	Blue	Orange
470-500	Blue - green	Red
500-520	Green	Purple
520-550	Yellow – green	Violet
550-580	Yellow	Violet- blue
580-620	Orange	Blue
620-680	Red	Blue- green
680-780	Purple	Green

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.

COLORIMETER:

A colorimeter is a device that is used in Colorimetry. It refers to a device which helps specific solutions to absorb a particular wavelength of light. The colorimeter is usually used to measure the concentration of a known solute in a given solution with the help of the Beer-Lambert law. The colorimeter was invented in the year 1870 by Louis J Duboscq. 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 themedium, temperature, order of mixing reagents, ageing of reagents and the careful assessment ofthe absorbance properties and stability of the chromophore generated Colorimetry has wide applications like study of molar composition of complexes, determination ofpKa value of indicators, determination of inorganic complexes and in quantitative analysis.Determination of formula and stability constants of the metal complexes is another importantapplication of visible spectrophotometry in addition to its quantitative analysis. The methods thatare commonly used for the optimization of colorimetric procedures and determination of formulaof the metal complex are Job's method of continuous variation, mole ratio method and Asmusmethod.



Principle of Colorimeter

It is a photometric technique which states that when a beam of incident light of intensity I_o passes through a solution, the following occur:

- A part of it is reflected which is denoted as I_r
- A part of it is absorbed which is denoted as I_a
- Rest of the light is transmitted and is denoted as I_t

Therefore, $I_o = I_r + I_a + I_t$

To determine I_a the measurement of I_o and I_t is sufficient therefore, I_r is eliminated. The amount of light reflected is kept constant to measure I_o and I_t.

Colorimeter is based on two fundamental laws of photometry. We have discussed them below:

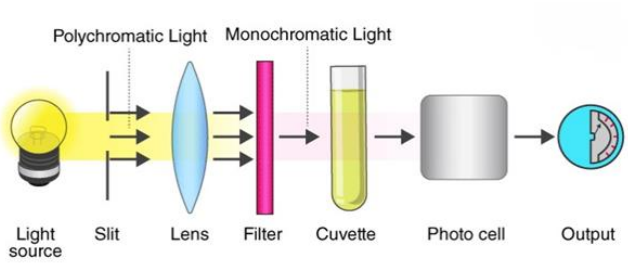
Beer's law:

According to this law the amount of light absorbed is proportional to the solute concentration present in solution.
$\text{Log}10\ I_o/I_t = a_s c$
where,
a_s is absorbency index
c is the concentration of solution

Lambert's law:

According to this law the amount of light absorbed is proportional to the length as well as thickness of the solution taken for analysis.
$A = \log 10\ I_o/I_t = a_s b$
Where,
A is the test absorbance of test
a_s is the standard absorbance
b is the length / thickness of the solution

Diagram of Colorimeter:



Parts of colorimeter:

1. Light Source: Produces light of a specific wavelength (color).
2. Monochromator: Filters the light to select a specific wavelength.
3. Sample Chamber: Holds the solution being measured.
4. Detector: Measures the intensity of light transmitted through the solution.
5. Display: Shows the absorbance reading.
6. Wavelength Selector: Allows selection of different wavelengths.
7. Cuvette: A small container holding the solution.
8. Optical Filter: Reduces stray light and improves accuracy.

Working of Colorimeter:

- Step 1: Before starting the experiment, it is important to calibrate the colorimeter. It is done by using the standard solutions of the known solute concentration that has to be determined. Fill the standard solutions in the cuvettes and place it in the cuvette holder of colorimeter.
- Step 2: A light ray of a certain wavelength, which is specific for the assay is in the direction of the solution. The light passes through a series of different lenses and filters. The coloured light navigates with

the help of lenses, and the filter helps to split a beam of light into different wavelengths allowing only the required wavelength to pass through it and reach the cuvette of the standard test solution.

- Step 3: When the beam of light reaches' cuvette, it is transmitted, reflected, and absorbed by the solution. The transmitted ray falls on the photodetector system where it measures the intensity of transmitted light. It converts it into the electrical signals and sends it to the galvanometer.
- Step 4: The electrical signals measured by the galvanometer are displayed in the digital form.
- Step 5: Formula to determine substance concentration in test solution.

$A = \epsilon cl$
For standard and test solutions
ϵ and l are constant
$A_T = C_T$ …. (i)
$A_S = C_S$ …. (ii)
From the above two equations,
$A_T \times C_S = A_S \times C_T$
$C_T = (A_T/A_S) \times C_S$
Where,
C_T is the test solution concentration
A_T is the absorbance/optical density of test solution
C_S is the standard concentration
A_S is the absorbance / optical density of standard solution.

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-marshal reagent
6. Dimedone
7. Deniges reagent
8. Dyes
9. Folin-coicalteu reagent
10. Froehde reagent

2,6-dichloroquinone-4-chloroimide:

Synonym: Gibb's reagent

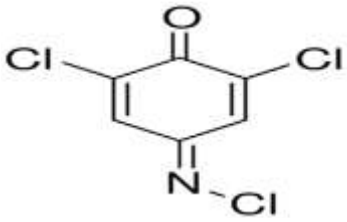


Figure 1: Structure of 2,6-dichloroquinone-4-chloroimide

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 correspondingproducts coupling with nucleophilic sites by elimination of chlorine.

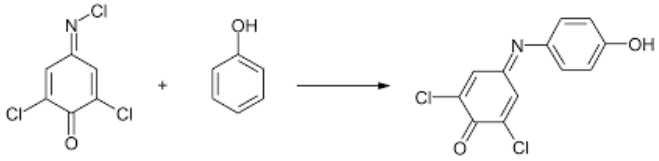


Figure 2: Reaction of gibb's reagent with phenols

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).
- 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).
- Bisoprolol fumarate: Drug is mixed with isopropanol and 5 mL of gibb's reagent is added and the mixture is heated (532 nm).
- 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).
- It is used in spectrophotometric determination of phenolic sympathomimetics like Ritodrine Hydrochloride.
- It is a very good reagent for determination of Vitamin B6.

- 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.

2,4-dinitrophenyl hydrazine:

Synonym: Brady's reagent

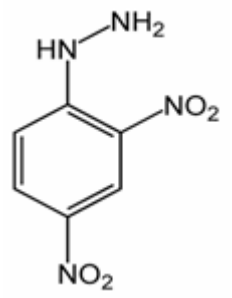
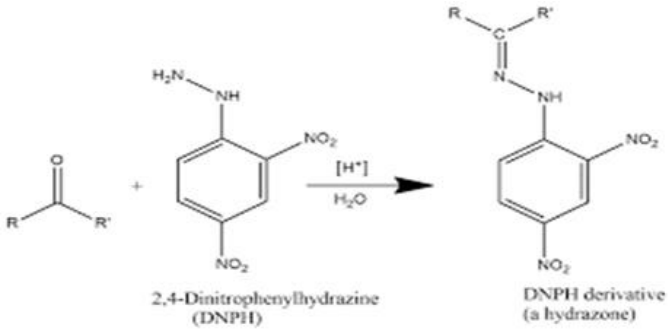


Figure 3: Structure of 2,4-dinitrophenyl hydrazine

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



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

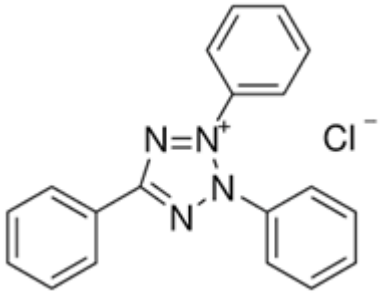


Figure 5: Structure of 2,3,5-triphenyl tetrazolium salt

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

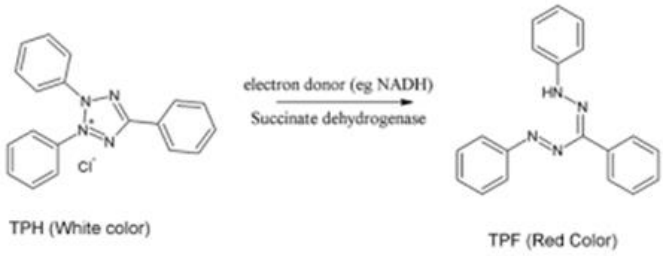


Figure 6: Reaction of 2,3,5-triphenyl tetrazolium salt

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)10.
- 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

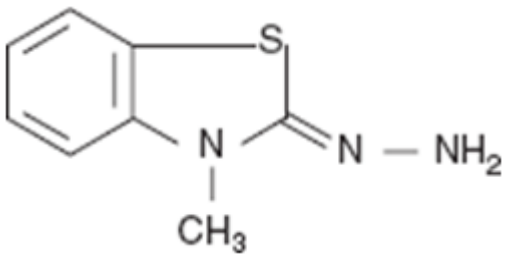


Figure 7: Structure of MBTH

Principle: Basic principle is oxidation followed by coupling. MBTH undergoes oxidative coupling reaction catalyzed 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.

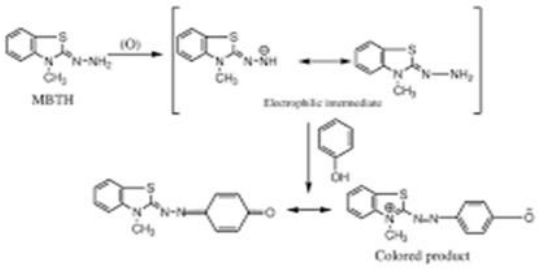


Figure8: Electrophilic intermediate of MBTH reagent

Applications: • Lamotrigine: Drug is dissolved in methanol, to each dilution 2 mL of MBTH and 2 mL FeCl3 are added, diluted with water which gave blue colour.

• Tenofovir: Drug is mixed with MBTH and this mixture produced apple green in presence of FeCl3 (626.5 nm).

• 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).

• Leukotriene: Drug is mixed with 1.5 mL MBTH and 2 mL FeCl3, kept aside and the absorbance of resulting green chromogen measured at 610m1.

Bratton-marshal reagent:
Synonym: Monomethanolate

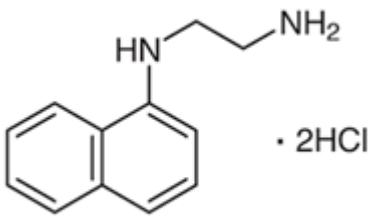


Figure 9: Structure of bratton-marshal reagent

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-marshal reagent produces violet chromogen (552 nm).

• Nimesulide: Drug is mixed with 0.1N hydrochloric acid and zinc dust, heated at 80oC and mixed with sodium nitrite, ammonium sulfamate and bratton marshal reagent, kept aside for 5 minutes (559 nm).

• 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 marshal reagent is added which produces pink chromogen (524 nm).

• Efavirenz: Drug is mixed with sodium nitrite, 2 mL of 2M hydrochloric acid and bratton marshal reagent, volume made up with water and kept aside produces purple chromogen (523 nm).

• Ceftazidime: Drug is mixed with 2 mL hydrochloric acid, 1 mL sulfamic acid and 1 mL bratton marshal reagent, left aside for 3 minutes and produces violet chromogen (575 nm).

• The other drugs estimated are Topiramate (551nm) and Amisulpride (530nm).

Dimedone:
Synonym: Cyclomethone

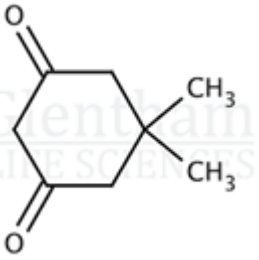


Figure 10: Structure of dimedone

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:

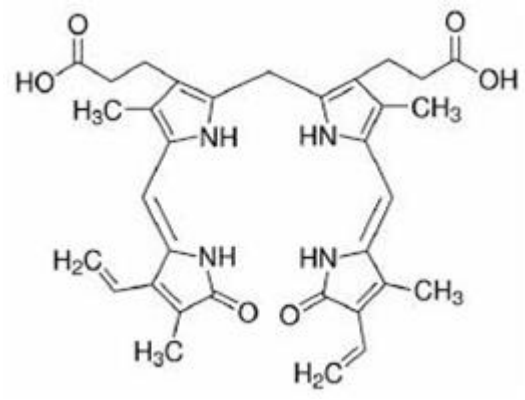
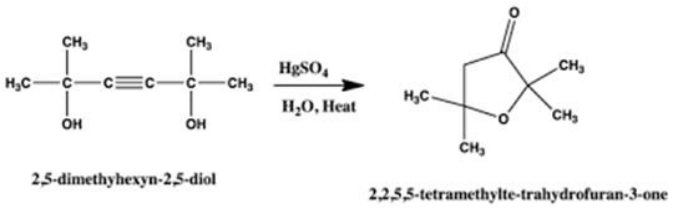


Figure 11: Structure of denige's reagent

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.



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 pH 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.

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).
- Telmisartan: Metanil yellow dye was used (420 nm).
- 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.

Folin-coicalteu reagent:
Synonym: Folin's phenol reagent:

Folin-Denis reagent It is a mixture of phosphomolybdtae 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:

$3H_2O_5.P_2O_5 13WO_3.5MoO3.10H_20$
$3H_2O.P_2O_5.14WO_3.4MoO3.10H_2O$

Principle: The main principle is reduction. When FC reagent reacts with the drug in presence of reducing agents like stannous chloride (SnCl2) 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).
- Ertapenem: Drug is mixed with sodium carbonate and FC reagent and the blue colour produced is measured at 848 nm.
- Flucloxacillin: Drug is mixed with 2 mL NaOH and 0.5mL FC reagent which produces pale blue colour (912 nm).
- 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.

- 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)
- 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.
- Amphetamine-type stimulants (ATS): Different ATS drugs like amphetamine, dmethamphetamine, phentermine, etc., were tested with this reagent and produced a variety of colors like pale yellow, light orange and light brown.
- 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.

- 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.

II. CONCLUSION

Colorimetric assays are tests that change color when an analyte (like a substance we want to measure) is present. These tests are commonly used in biochemistry to detect enzymes, hormones, and other compounds.

In the pharmaceutical field, colorimetric methods can help determine the number of drugs in various forms, including tablets and biological samples, using spectrophotometry and chromatography. These methods are very sensitive and often work better than UV spectroscopy because they measure absorbance at longer wavelengths, reducing interference from other ingredients.

Additionally, colorimetric tests are usually inexpensive and the necessary reagents are easy to find. There is also plenty of opportunity to create new reagents, making these methods useful for routine drug analysis across many different types of samples.