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DEVELOPMENT OF NOVEL IDENTIFICATION TESTS FOR ALKALOIDS

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ABSTRACT

Alkaloids exhibit diversity with respect to chemical structure and physiological activity. In general, four precipitating tests are popular for detection of alkaloids. The chemical tests for identification of Atropine sulphate, Strychnine hydrochloride and Brucine are a few. In the present study, 12 novel identification tests each for identification of Atropine sulphate, Strychnine hydrochloride and Brucine have been developed. The tests are developed for the alkaloids dissolved in alcohol or distilled water using oxidizing agents Potassium dichromate/Potassium permanganate/Ceric ammonium sulphate in ortho phosphoric acid or lactic acid. These tests depend upon the generation of oxygen in an acid solution of the alkaloid. The identification of the alkaloid is both the initial colour produced, as well as the sequence of colour changes occurring with time. The developed tests are found to be relatively simple, economic, stable and sensitive compared to the so far reported general alkaloid tests and specific alkaloid tests for the identification of Atropine sulphate, Strychnine hydrochloride and Brucine.

INTRODUCTION:

Alkaloids exhibit diversified pharmacological actions. They are obtained from natural or synthetic origin, basic in nature, generally bitter in taste. Alkaloids contain one or more nitrogen atoms normally in a heterocyclic ring [1]. In nature, alkaloids exist either as free bases or salts with acids or alkaloid N-oxides. The free bases are insoluble in water but soluble in most of the organic solvents. Most of the alkaloids are levorotatory, some are dextrorotatory and some more are optically inactive [2]. Atropine is a tropane alkaloid, present in Atropa belladonna, Hyoscyamus muticans and Hyoscyamus niger (Solanaceae) etc. Atropine decreases oropharyngeal, tracheal and bronchial secretions and relieves associated bronchospasm and bronchoconstriction. Atropine is an anticholinergic, used to decrease salivary and gastric secretions. Atropine sulphate is used as preanesthetic medication before surgery to decrease the salivary and respiratory secretions during surgery. It is an antispasmodic, used to treat spasms in the stomach, intestines, urinary tract, and other smooth muscles. Atropine acts as an antidote in opium, chloralhydrate, muscarinic mushroom poisoning and organophosphate poisoning [3-5]. Atropine also reduces central respiratory paralysis caused by anticholinesterases. It is used to restore arterial blood pressure and heart rate during anesthesia when vagal stimulation produced by intra-abdominal surgical traction causes decrease in pulse rate and cardiac function. Used to decrease digitalis induced atrioventricular block, when increased vagal tone is a major factor in the conduction defect. Used to treat hyperactive

carotid sinus reflex induced bradycardia and syncope. Atropine treats bradycardia by increasing the heart rate and thereby improving atrioventricular conduction through blocking the parasympathetic influences on the heart [6-7].

Strychnine and Brucine are indole alkaloids. Strychnine and Brucine are present in the seeds of Strychnos nuxvomica L. beans of Strychnos ignatti Berg. roots of S. cinnamomifolia Thw. seeds, bark and wood of S. colubrina Linn, and plant of S. malaccensis Benth (Loganiaceae) etc [8]. The alkaloids act on the nervous system, exhibit strong sedative and tranquilizing effect. Strychnine is a powerful CNS stimulant, Brucine closely resembles strychnine in physiological action but is relatively slower in action and is readily eliminated from body. In Homeopathy medicine, strychnine is used to treat atonic dyspepsia, constipation, heart burn and indigestion. Chinese herbalists use Nuxvomica externally to treat tumors, headaches and paralysis. Strychnine is used in conjunction with antivenoms to treat poisonous snake bites. Strychnine is used as a poison baits for rodents [9-11]. Chemical tests for alkaloids are of two types. 1. General tests 2. Specific tests.

`General tests are the qualitative chemical tests used for the detection of alkaloids and depend on either precipitation reactions or color formation reactions. Alkaloid precipitating reagents are used for knowing the presence or absence of alkaloids in a substance. The limitation with the reagents is some of the nitrogen containing non-alkaloid substances such as proteins, betaines and ammonium salts also give false positive results. Though in the following table specific color precipitates are displayed for different general alkaloid tests, some alkaloids show completely distinct color than mentioned in the table -1 [12-13].

1. Precipitating reagents: These reagents are further classified as follows

1. Reagents that form double salts: Dragendorff's, Modified dragendorff's, Mayer's, Marme's and Gold chloride
2. Reagents Containing Halogens: Wagner's
3. Organic Acids: Hager's, Tannic Acid and Picrolonic acid
4. Oxygenated High Molecular Weight Acids: Phosphomolybdic acid (Sonnenschein's), Phosphotungstic acid (Scheibler's) and Silicotungstic acid

2. Colour reactions of Alkaloids

Reactions of alkaloids with most of the colour forming reagents are useful in detection of alkaloids even in traces. Generally, the colour reactions of alkaloids are based on dehydration or oxidation. Most of these reagents mainly consist of conc. Sulphuric acid along with compounds, such as, sulphomolybdic acid, formaldehyde, sulphovanadic acid, potassium arsenate, hydrogen peroxide, and selenious acid. These reactions are useful to know the presence of type of alkaloids, but not useful in confirming specific alkaloid. In many reactions, the composition of the color formed is not known. In some of these color reactions, the intensity of color produced is linear. So, these reactions are used for the quantitative estimation of alkaloids. For example, the blue color produced with Van Urk's reagent or Ehrlich reagent is used for quantitative estimation of indole alkaloids mainly tryptamines and ergot alkaloids. The violet color produced by tropane alkaloids with Vitali Morin's reagent is used for quantitative estimation of tropane alkaloids. Though some of the alkaloids in a class exhibit similar color, some more show distinct colors with the same color forming reagent [14-16].

3. Specific tests

Other reagents are available to identify specific alkaloids, mainly those that give color reactions of subgroups of alkaloids. The specific tests for individual alkaloids are more important for qualitative evaluation of alkaloids.

Atropine sulphate: Atropine is a tropane alkaloid. Atropine sulphate gives reactions of alkaloids and sulphates.

1. Gerrard Reaction: About 0.1 gram of Atropine dissolved in 2 ml of alcohol, treated with 2ml of 2%w/v mercuric chloride in 50% ethanol forms an instant red color mercuric oxide.

2. Test with Acids: Atropine with conc. Sulphuric acid should not produce any color and should not develop any color by the subsequent addition of nitric acid [13,17].

3. Schaer's Reagent: Hyoscyamine produces green color with a few drops of 1ml of 30% H_2O_2 mixed with 10ml of conc. Sulphuric acid.

4. Vitali-Morin's Reaction: The reaction is useful for the detection of tropane alkaloids. When Atropine is treated with fuming HNO_3 followed by evaporation to dryness on a water-bath forms polynitrocompound of yellow color. The yellow color compound dissolved in acetone on treatment with freshly prepared 0.5 ml of a 3%w/v solution of KOH in methanol shows violet red color slowly changes to red.

5. *para*-Dimethylaminobenzaldehyde Reagent (Van Urk's reagent): The reagent is used for ergot alkaloids and pyrrolizidine alkaloids identification. Indole alkaloids show blue color with the reagent. Hyoscyamine upon treatment with 2-3 drops of *p*-Dimethylaminobenzaldehyde reagent and heating on a boiling water-bath for several minutes forms a distinct red color that ultimately changes to permanent cherry red upon cooling.

6. Picric acid reagent: About 50 mg of Atropine sulphate in 5 ml of water, with 5 ml of picric acid scolution forms yellow precipitate. The precipitate, when washed with water, then dried at 100 ℃ to 105 ℃ for 2 hours, should melt at 174 ℃ to 179 ℃ [4, 18-20].

Strychnine hydrochloride and Brucine: 1. **Otto reaction:** Solution of strychnine in 80% sulphuric acid and potassium dichromate solution gives a reddish violet to bluish purple color.

2. Ferric chloride test: Strychnine and most of its derivatives in weakly acid solutions with ferric chloride show bright red color. Brucine with nitric acid gives intense orange red color [21].

3. Potassium dichromate-Sulphuric acid test: Strychnine solution on addition of dilute solution of potassium dichromate forms yellow crystalline precipitate, which upon washing and heating with conc. Sulphuric acid shows initially brilliant violet color then turns to cherry red to rose pink finally to yellow [22].

4. Sulphuric acid-Dichromate Test: Except Strychnine nitrate, all strychnine derivatives give the test. The most distinctive test for strychnine is treatment with conc. Sulphuric acid and oxidizing agents. With cold Sulphuric acid, pure strychnine gives a colorless solution. The U. S. P. color test, for strychnine involves dissolving minute amount in 0.5ml of conc. Sulphuric acid, and dropping a small crystal of potassium dichromate slowly in the liquid with a glass rod. Initially produces a blue color, turns to purplish-blue, then gradually to violet, purplish-red, cherry-red, and finally to orange or yellow. The blue color in the strychnine test is also obtained using oxidizing agents such as dioxide of manganese (MnO2), of lead (PbO2) and potassium permanganate.

5. Mandelin's Reagent: Strychnine or its salts when treated with a saturated solution of ammonium vanadate, produces purple color.

6. Nitric acid Test: Strychnine on treatment with a trace of conc. HNO_3 yields an instant yellow color. Brucine gives an intense orange-red color and may be used to differentiate strychnine from brucine [8-23].

7. Van-Urk's reagent: Indole alkaloids give blue color with p-dimethyl amino benzaldehyde [18]. From literature review, research on development of identification tests for alkaloids is not focused well. Most

of the reported general and specific tests for the detection of alkaloids are costly and time taking. So far, developed general tests for the detection of alkaloids and most of the specific tests for identification of Atropine sulphate, Strychnine Hydrochloride and Brucine are complex. Therefore, the present study is focused on development of alternative identification tests for the detection of pharmaceutically important alkaloids such as Atropine sulphate, Strychnine hydrochloride and Brucine.

MATERIALS AND METHODS

Apparatus: Test tubes**,** beakers**,** measuring jars, pipettes, test tube stands, glass rods and electronic balance.

Chemicals

Atropine sulphate- LOBA CHEMIE private limited, Mumbai, India. Brucine- Sd. Fine Chem limited, Mumbai, India. Strychnine hydrochloride- Deccan Phyto Chemicals, Pattancheru. Distilled water, Ethyl alcohol A.R. 99.9%. Potassium dichromate- Qualigens Fine Chemicals, Mumbai. Potassium permanganate- Sd. Fine Chem limited, Mumbai, India. Ceric ammonium sulphate- Sd. Fine Chem limited, Mumbai, India. Ortho phosphoric acid 70%w/w AR - Merck specialties private limited, Mumbai, India. Lactic acid 90%w/w Extra pure- Merck specialties private limited, Mumbai, India.

Test Solutions: 1%w/v atropine sulphate in distilled water, 1% w/v atropine sulphate in alcohol, 1% w/v strychnine hydrochloride in distilled water, 1% w/v strychnine hydrochloride in alcohol, 1% w/v brucine in distilled water, 1% w/v brucine in alcohol.

Reagents: 1%w/v potassium dichromate solution, 0.1 N ceric ammonium sulphate solution and 0.1N potassium permanganate solution.

GENERAL PROCEDURE:

- About 1ml of 1%w/v Atropine sulphate/Strychnine hydrochloride/Brucine prepared in distilled water is transferred in to a test tube and 1ml of 1%w/v potassium dichromate is added mixed thoroughly and observed for color changes. To the mixture about 1ml of 70% w/w ortho phosphoric acid was added and the color changes were noted (Table-3-5 and figure-1, 3, 5).
- About 1ml of 1%w/v Atropine sulphate/Strychnine hydrochloride/Brucine prepared in alcohol is transferred in to a test tube and 1ml of 1%w/v potassium dichromate is added mixed thoroughly and observed for color changes. To the mixture 1ml of 70% ortho phosphoric acid was added and the color changes were noted (Table-3-5 and figure-2, 4, 6).
- About 1ml of 1%w/v Atropine sulphate/Strychnine hydrochloride/Brucine prepared in distilled water is transferred in to a test tube and 1ml of 1%w/v potassium dichromate is added mixed thoroughly and observed for color changes. To the mixture about 1ml of 90%w/w lactic acid was added and the color changes were noted (Table-3-5 and figure-1, 3, 5).
- About 1ml of 1%w/v Atropine sulphate/Strychnine hydrochloride/Brucine prepared in alcohol is transferred in to a test tube and 1ml of 1%w/v potassium dichromate is added mixed thoroughly and observed for color changes. To the mixture 1ml of 90% lactic acid was added and the color changes were noted (Table-3-5 and figure-2, 4, 6).
- About 1ml of 1%w/v Atropine sulphate/Strychnine hydrochloride/Brucine prepared in distilled water is transferred in to a test tube and 1ml of 0.1N potassium permanganate was added mixed thoroughly and observed for color changes. To the mixture about 1ml of 70% ortho phosphoric acid was added and the color changes were noted (Table-3-5 and figure-1, 3, 5).
- About 1ml of 1%w/v Atropine sulphate/Strychnine

hydrochloride/Brucine prepared in alcohol is transferred in to a test tube and 1ml of 0.1N potassium permanganate was added mixed thoroughly and observed for color changes. To the mixture 1ml of 70% ortho phosphoric acid was added and the color changes were noted (Table-3-5 and figure-2, 4, 6).

- About 1ml of 1%w/v Atropine sulphate/Strychnine hydrochloride/Brucine prepared in distilled water is transferred in to a test tube and 1ml of 0.1N potassium permanganate was added mixed thoroughly and observed for color changes. To the mixture about 1ml of 90% lactic acid was added and the color changes were noted (Table-3-5 and figure-1, 3, 5).
- About 1ml of 1%w/v Atropine sulphate/Strychnine hydrochloride/Brucine prepared in alcohol is transferred in to a test tube and 1ml of 0.1N potassium permanganate was added mixed thoroughly and observed for color changes. To the mixture 1ml of 90% lactic acid was added and the color changes were noted (Table-3-5 and figure-2, 4, 6).
- About 1ml of 1%w/v Atropine sulphate/Strychnine hydrochloride/Brucine prepared in distilled water is transferred in to a test tube and 1ml of 0.1N ceric ammonium sulphate was added mixed thoroughly and observed for color changes. To the mixture about 1ml of 70% ortho phosphoric acid was added and the color changes were noted (Table-3-5 and figure-1, 3, 5).
- About 1ml of 1%w/v Atropine sulphate/Strychnine hydrochloride/Brucine prepared in alcohol is transferred in to a test tube and 1ml of 0.1N of 0.1N ceric ammonium sulphate was added mixed thoroughly and observed for color changes. To the mixture 1ml of 70% ortho phosphoric acid was added and the color changes were noted (Table-3-5 and figure-2, 4, 6).
- About 1ml of 1%w/v Atropine sulphate/Strychnine hydrochloride/Brucine prepared in distilled water is transferred in to a test tube and 1ml of 0.1N ceric ammonium sulphate was added mixed thoroughly and observed for color changes. To the mixture about 1ml of 90% lactic acid was added and the color changes were noted (Table-3-5 and figure-1, 3, 5).
- About 1ml of 1%w/v Atropine sulphate/Strychnine hydrochloride/Brucine prepared in alcohol is transferred in to a test tube and 1ml of 0.1N of 0.1N ceric ammonium sulphate was added mixed thoroughly and observed for color changes. To the mixture 1ml of 90% lactic acid was added and the color changes were noted (Table-3-5 and figure-2, 4, 6).

DISCUSSION

To date many tests are reported for the identification of alkaloids. But chemical tests for the identification of Atropine sulphate, Strychnine hydrochloride and Brucine are limited. In the present study, 12 identification tests each, for Atropine sulphate, Strychnine hydrochloride and Brucine using distilled water or alcohol as solvent are developed using oxidizing agents such as 1%w/v Potassium dichromate/ 0.1N Potassium permanganate or 0.1N Ceric ammonium sulphate in acid media like Orthophosphoric acid or Lactic acid. Mandelin's test for strychnine depends upon the generation of oxygen in conc. Sulphuric acid solution of the alkaloid. For the generation of oxygen manganese dioxide, Cerium oxide, Lead peroxide, Potassium dichromate, Potassium ferrocyanide, Ammonium vanadate may be used [24]. Orthophosphoric acid and its salts are powerful reducing agents, the free acid decomposing into phosphine, a powerful reducing agent [25]. When Potassium dichromate is used with a reducing agent the orange solution containing dichromate $Cr_2O_7^{-2}$ (Cr^{6+}) ions gets reduces to a green solution containing (Cr^{3+}) [26].

Atropine sulphate in distilled water and Strychnine hydrochloride in distilled water treated with 1%w/v Potassium dichromate in ortho phosphoric acid produced yellow orange color. So, 1%w/v Potassium dichromate in ortho phosphoric acid could be used as a common test for the identification of Atropine sulphate and Strychnine hydrochloride in distilled water. Whereas, in the test when the alkaloids are dissolved in alcohol emerald green color with Atropine sulphate and chlorophyll green color with Strychnine hydrochloride observed indicating that the color formed in the test is influenced by the solvent used for dissolving the alkaloids. Probably the reason for the green color formation in the test is due to the reduction of orange color dichromate to green color Cr^{3+} by ortho phosphoric acid in the presence of alkaloid. The reason for similar color formation with Atropine sulphate and Strychnine hydrochloride in alcohol treated with the reagent is to be further explored. Atropine sulphate in distilled water or alcohol and Strychnine hydrochloride in alcohol treated with 1%w/v Potassium dichromate and lactic acid showed greenish blue. So, the reagent Potassium dichromate and lactic acid could be used as a common test to detect Atropine sulphate in water as well as in alcohol and Strychnine hydrochloride in alcohol. Strychnine hydrochloride in distilled water treated with 1%w/v Potassium dichromate or 0.1N Potassium permanganate and lactic acid formed brown color indicating that the alkaloid could be identified using either of the oxidizing agents with lactic acid. In the test, the color produced is influenced by the solvent used to dissolve the alkaloid. May be the mechanism involved is oxidation of alkaloid in the presence of Potassium dichromate and lactic acid. Atropine sulphate/Strychnine hydrochloride in distilled water or alcohol treated with 0.1N Potassium permanganate and orthophosphoric acid produced purple color. So, Potassium permanganate and orthophosphoric acid could be used as a common test for identification of Atropine sulphate/Strychnine hydrochloride in distilled water or alcohol as there is no influence of solvent on the color formation. Atropine sulphate/Strychnine hydrochloride/Brucine in distilled water or alcohol treated with 0.1N Potassium permanganate and lactic acid produced brown color. So, the test could be used as a common test to identify Atropine sulphate, Strychnine hydrochloride and Brucine using either water or alcohol as solvent as there is no influence of solvent on the color formation.

Similarity also observed between Atropine sulphate in alcohol and Strychnine hydrochloride in alcohol treated with 0.1N Ceric ammonium sulphate and ortho phosphoric acid/lactic acid. Both the alkaloids produced colorless glossy gel in the test. So, 0.1N Ceric ammonium sulphate and ortho phosphoric acid/lactic acid could be used as a common test for the identification of Atropine sulphate in alcohol and Strychnine hydrochloride in alcohol. The reason for similar color formation with Atropine sulphate and Strychnine hydrochloride in alcohol with this reagent is to be further explored. Atropine sulphate in distilled water and Strychnine hydrochloride in distilled water treated with Ceric ammonium sulphate and lactic acid showed pale yellow color indicating that Ceric ammonium sulphate and lactic acid could be used as common reagents for the identification of Atropine sulphate or Strychnine hydrochloride dissolved in distilled water. Brucine in alcohol with 0.1N $KMnO_4$ and orthophosphoric acid produced orange-red color but with lactic acid brownish shade observed. Brucine in alcohol with 0.1N Ceric ammonium sulphate and ortho phosphoric acid/Lactic acid developed intense orange-red color. Whereas, Atropine sulphate/Strychnine hydrochloride dissolved in alcohol or water treated with 0.1N Ceric ammonium sulphate and ortho phosphoric acid/Lactic acid resulted in white glassy color. So, the developed test with 0.1N Ceric ammonium sulphate and ortho phosphoric acid/Lactic acid or 0.1N $KMnO_4$ and orthophosphoric acid could be used as a differentiating test for Brucine from Atropine sulphate or Strychnine hydrochloride.

Table.1: Precipitating reagents

Reagent name	Reagent composition	Color ppt formed
Dragendorff's	Potassium-bismuth-iodide Solution (basic bismuth nitrate, tartaric acid and KI)	Orange
Kraut's/Modified dragendorff's	Bismuth subnitrate in 20% glacial acetic acid, 40% aqueous solution of KI and glacial acetic acid (Potassium bismuth iodide)	Bright Brick red
Mayer's	Potassium-mercuric-iodide solution	Cream
Marme's	Cadmium iodide and KI in conc. H_2SO_4 (Potassium Cadmium Iodide)	Yellow
Gold chloride	Auric chloride Au_2Cl_6	Yellow
Wagner's	I_2 in KI solution	Brown
Hager's	Saturated solution of picric acid	Yellow
Tannic acid	Tannic acid	Buff
Picrolonic acid	Picrolonic acid (I-p-nitro-phenyl-3methyl-4-nitro-5-pyrazolon)	Yellow
Sonnenschein's	(Phosphomolybdic Acid) in dil HNO_3	Yellowish turns blue or green (reduction of molybdic acid)
Scheiblers	Sodium Tungstic acid and Disodium Phosphate (Phosphotungstic acid)	Characteristic color
Bertrand's	(Silicotungstic acid)	Characteristic color
Reineckate salt solution:	Ammonium Reineckate and Hydroxylamine HCl	Characteristic color

Table.2: Color formation reactions

Color forming reagent name	Reagent composition	Color formed
Vitali Morin's	Conc. HNO_3, Acetone, Alcoholic KOH,	Violet
Marquis	40% Formaldehyde solution in conc. H_2SO_4	Blood-red
Van Urck's /Ehrlich's	p-dimethylaminobenzaldehyde, ethanol and conc. HCl	Blue
Mandelin's	Ammonium metavanadate in conc. H_2SO_4 (Sulphovanidic acid)	Violet
Erdmann's	Mixture of conc. H_2SO_4 and conc. HNO_3	Red
Froehde's (freshly prepared)	Sodium molybdate in conc. H_2SO_4 (Phosphomolybdic acid)	Red, blue, green
Vasitsky's	p-dimethylaminobenzaldehyde in conc. H_2SO_4	Violet
Schaer's (freshly prepared)	30% H_2O_2 and conc. H_2SO_4	Green
Mecke's	Selenious acid in conc. H2SO4	Characteristic color
Rosenthaler's	Potassium arsenate in conc. H_2SO_4	Characteristic color

Table No. 3: Identification tests for Atropine sulphate

S.No./Code	Test substance	Reagent	Result
1. (1AW)	1ml 1%w/v Atropine sulphate in distilled water	1ml 1%w/v$K_2Cr_2O_7$ + (1ml) 70%Ortho Phosphoric acid	Initially with potassium dichromate yellow color produced, upon addition of ortho phosphoric acid no change in color observed.
2. (1AA)	1ml 1%w/v Atropine sulphate in alcohol	1ml 1%w/v $K_2Cr_2O_7$ + (1ml) 70%Ortho Phosphoric acid	Initially with potassium dichromate yellow color produced, upon addition of ortho phosphoric acid greenish yellow color observed after 10minutes the color turned to emerald color.
3. (2AW)	1ml 1%w/v Atropine sulphate in distilled water	1ml 1%w/v $K_2Cr_2O_7$ + (1ml) 90% Lactic acid	Initially with potassium dichromate yellow color produced, upon addition of lactic acid greenish blue color observed.
4. (2AA)	1ml 1%w/v Atropine sulphate in alcohol	1ml 1%w/v $K_2Cr_2O_7$ + (1ml) 90% Lactic acid	Initially with potassium dichromate yellow color produced, upon addition of lactic acid pale green color observed after 10minutes changed to greenish blue.
5. (3AW)	Atropine sulphate in distilled water	1ml 0.1N $KMnO_4$+ (1ml) 70%Ortho Phosphoric acid	Initially with potassium permanganate dark purple color produced, upon addition of ortho phosphoric acid the color turned to pale purple.
6. (3AA)	1ml 1%w/v Atropine sulphate in alcohol	1ml 0.1N $KMnO_4$+ (1ml) 70%Ortho Phosphoric acid	Initially with potassium permanganate dark purple color produced, upon addition of ortho phosphoric acid the color turned to pale purple.
7. (4AW)	1ml 1%w/v Atropine sulphate in distilled water	1ml 0.1N $KMnO_4$+ (1ml) 90% Lactic acid	Initially with potassium permanganate dark purple color produced, upon addition of lactic acid purplish brown color observed.
8. (4AA)	1ml 1%w/v Atropine sulphate in alcohol	0.1N (1ml) $KMnO_4$+ (1ml) 90% Lactic acid	Initially with potassium permanganate dark purple color produced, upon addition of lactic acid brown color observed.
9. (5AW)	1ml 1%w/v Atropine sulphate in distilled water	1ml 0.1N Ceric ammonium sulphate+ (1ml) 70%Ortho Phosphoric acid	Initially with ceric ammonium sulphate yellow color produced, upon addition of ortho phosphoric acid colorless glossy gel observed.
10. (5AA)	1ml 1%w/v Atropine sulphate in alcohol	1ml 0.1N Ceric ammonium sulphate+ (1ml) 70%Ortho Phosphoric acid	Initially with ceric ammonium sulphate yellow color produced, upon addition of ortho phosphoric acid colorless glossy gel observed.
11. (6AW)	1ml 1%w/v Atropine sulphate in distilled water	1ml 0.1N Ceric ammonium sulphate+(1ml) 90% Lactic acid	Initially with ceric ammonium sulphate yellow color produced, upon addition of lactic acid pale yellow gel formed.
12. (6AA)	1ml 1%w/v Atropine sulphate in alcohol	1ml 0.1N Ceric ammonium sulphate+(1ml) 90% Lactic acid	Initially with ceric ammonium sulphate yellow color produced, upon addition of lactic acid colorless glossy gel observed.

***AW= Atropine sulphate in distilled water: AA= Atropine sulphate in alcohol**

Table No. 4: Identification tests for Strychnine hydrochloride

S.No./Code	Test substance	Reagent	Result
1. (1SW)	1ml 1%w/v Strychnine hydrochloride in distilled water	1ml 1%w/v $K_2Cr_2O_7$ + (1ml) 70%Ortho Phosphoric acid	Initially with potassium dichromate yellow orange precipitate formed and the precipitate disappeared on addition of ortho Phosphoric acid.
2. (1SA)	1ml 1%w/v Strychnine	1ml 1%w/v $K_2Cr_2O_7$ + (1ml) 70%Ortho	Initially with potassium dichromate yellow orange color formed, upon

	hydrochloride in alcohol	Phosphoric acid	addition of ortho Phosphoric acid the color turned to Chlorophyll green.
3. (2SW)	1ml 1%w/v Strychnine hydrochloride in distilled water	1ml 1%w/v $K_2Cr_2O_7$ + (1ml) 90% Lactic acid	Initially with potassium dichromate yellow orange precipitate formed, upon addition of lactic acid brown color observed.
4. (2SA)	Strychnine hydrochloride in alcohol	1ml 1%w/v $K_2Cr_2O_7$ + (1ml) 90% Lactic acid	Initially with potassium dichromate yellow orange color produced, upon addition of lactic acid pale green color observed after 10minutes changed to greenish blue.
5. (3SW)	1ml 1%w/v Strychnine hydrochloride in distilled water	0.1N (1ml) $KMnO_4$+ (1ml) 70%Ortho Phosphoric acid	Initially with potassium permanganate dark purple color produced, upon addition of ortho phosphoric acid the color turned to pale purple.
6. (3SA)	Strychnine hydrochloride in alcohol	1ml 0.1N $KMnO_4$+ (1ml) 70%Ortho Phosphoric acid	Initially with potassium permanganate dark purple color produced, upon addition of ortho phosphoric acid the color turned to pale purple.
7. (4SW)	1ml 1%w/v Strychnine hydrochloride in distilled water	1ml 0.1N $KMnO_4$+ (1ml) 90% Lactic acid	Initially with potassium permanganate dark purple color produced, upon addition of lactic acid brown color observed.
8. (4SA)	Strychnine hydrochloride in alcohol	1ml 0.1N $KMnO_4$+ (1ml) 90% Lactic acid	Initially with potassium permanganate dark purple color produced, upon addition of lactic acid purplish brown color observed.
9. (5SW)	1ml 1%w/v Strychnine hydrochloride in distilled water	1ml 0.1N Ceric ammonium sulphate+ (1ml) 70%Ortho Phosphoric acid	Initially with ceric ammonium sulphate yellow color produced, upon addition of ortho phosphoric acid colorless glossy gel observed.
10. (5SA)	1ml 1%w/v Strychnine hydrochloride in alcohol	1ml 0.1N Ceric ammonium sulphate+ (1ml) 70%Ortho Phosphoric acid	Initially with ceric ammonium sulphate yellow color produced, upon addition of ortho phosphoric acid colorless glossy gel observed.
11. (6SW)	1ml 1%w/v Strychnine hydrochloride in distilled water	1ml 0.1N Ceric ammonium sulphate+ (1ml) 90% Lactic acid	Initially with ceric ammonium sulphate yellow color produced, upon addition of lactic acid yellow color gel observed.
12. (6SA)	1ml 1%w/v Strychnine hydrochloride in alcohol	1ml 0.1N Ceric ammonium sulphate+ (1ml) 90% Lactic acid	Initially with ceric ammonium sulphate yellow color produced, upon addition of lactic acid colorless glossy gel observed.

***SW= Strychnine hydrochloride in distilled water: SA= Strychnine hydrochloride in alcohol**

Table No. 5: Identification tests for Brucine

S.No./Code	Test substance	Reagent	Result
1. (1BW)	1ml 1%w/v Brucine in distilled water	1ml 1%w/v $K_2Cr_2O_7$ + (1ml) 70%Ortho Phosphoric acid	Initially with potassium dichromate yellow color precipitate formed, upon addition of ortho Phosphoric acid orangish red color observed.
2. (1BA)	1ml 1%w/v Brucine in alcohol	1ml 1%w/v $K_2Cr_2O_7$ + (1ml) 70%Ortho Phosphoric acid	Initially with potassium dichromate yellow color formed, upon addition of ortho Phosphoric acid orangish

			red color observed.
3. (2BW)	1ml 1%w/v Brucine in distilled water	1ml 1%w/v $K_2Cr_2O_7$ + (1ml) 90% Lactic acid	Initially with potassium dichromate yellow color precipitate formed, upon addition of lactic acid brown red color observed.
4. (2BA)	1ml 1%w/v Brucine in alcohol	1ml 1%w/v $K_2Cr_2O_7$ + (1ml) 90% Lactic acid	Initially with potassium dichromate yellow color formed, upon addition of lactic acid brown color observed.
5. (3BW)	1ml 1%w/v Brucine in distilled water	1ml 0.1N $KMnO_4$+ (1ml) 70%Ortho Phosphoric acid	Initially with potassium permanganate brown color produced, upon addition of ortho phosphoric acid the color turned to orange red color.
6. (3BA)	1ml 1%w/v Brucine in alcohol	1ml 0.1N $KMnO_4$+ (1ml) 70%Ortho Phosphoric acid	Initially with potassium permanganate dark purple color produced, upon addition of ortho phosphoric acid the color turned orange color.
7. (4BW)	1ml 1%w/v Brucine in distilled water	1ml 0.1N $KMnO_4$+ (1ml) 90% Lactic acid	Initially with potassium permanganate dark purple color produced, upon addition of lactic acid brown color observed.
8. (4BA)	1ml 1%w/v Brucine in alcohol	1ml 0.1N $KMnO_4$+ (1ml) 90% Lactic acid	Initially with potassium permanganate dark purple color produced, upon addition of lactic acid brown color observed.
9. (5BW)	1ml 1%w/v Brucine in distilled water	1ml 0.1N Ceric ammonium sulphate+ (1ml) 70%Ortho Phosphoric acid	Initially with ceric ammonium sulphate yellowish red color produced, upon addition of ortho phosphoric acid peach color gel observed.
10. (5BA)	1ml 1%w/v Brucine in alcohol	1ml 0.1N Ceric ammonium sulphate+ (1ml) 70%Ortho Phosphoric acid	Initially with ceric ammonium sulphate yellowish red color produced, upon addition of ortho phosphoric acid orange red color observed.
11. (6BW)	1ml 1%w/v Brucine in distilled water	1ml 0.1N Ceric ammonium sulphate+ (1ml) 90% Lactic acid	Initially with ceric ammonium sulphate yellowish red color produced, upon addition of lactic acid yellowish orange gel observed.
12. (6BA)	1ml 1%w/v Brucine in alcohol	1ml 0.1N Ceric ammonium sulphate+ (1ml) 90% Lactic acid	Initially with ceric ammonium sulphate yellowish red color produced, upon addition of lactic acid pale orange gel observed.

***BW= Brucine in distilled water: BA= Brucine in alcohol**

Figure-1: Identification tests for Atropine sulphate in distilled water (AW)

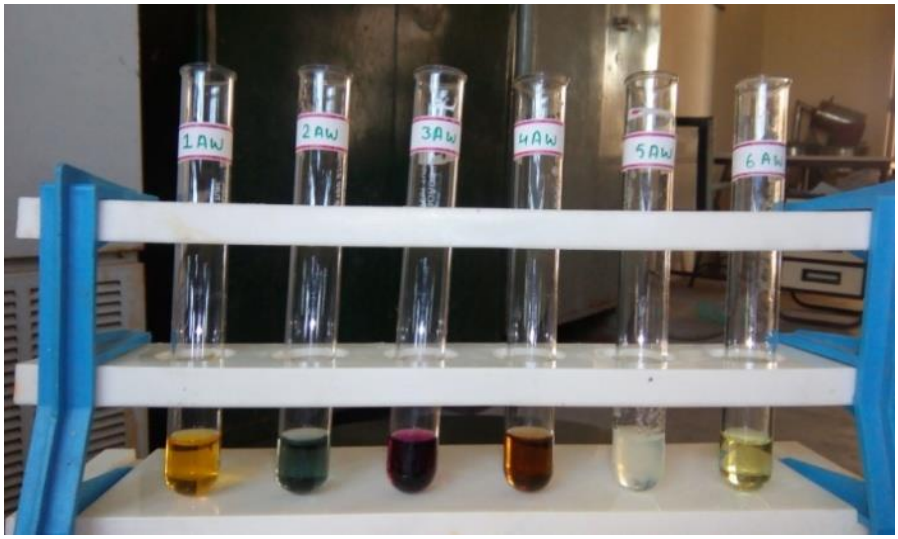

Figure-2: Identification tests for Atropine sulphate in alcohol (AA)

Figure-3: Identification tests for Strychnine hydrochloride in distilled water (SW)

Figure-4: Identification tests for Strychnine hydrochloride in Alcohol (SA)

Figure-5: Identification tests for Brucine in distilled water- (BW)

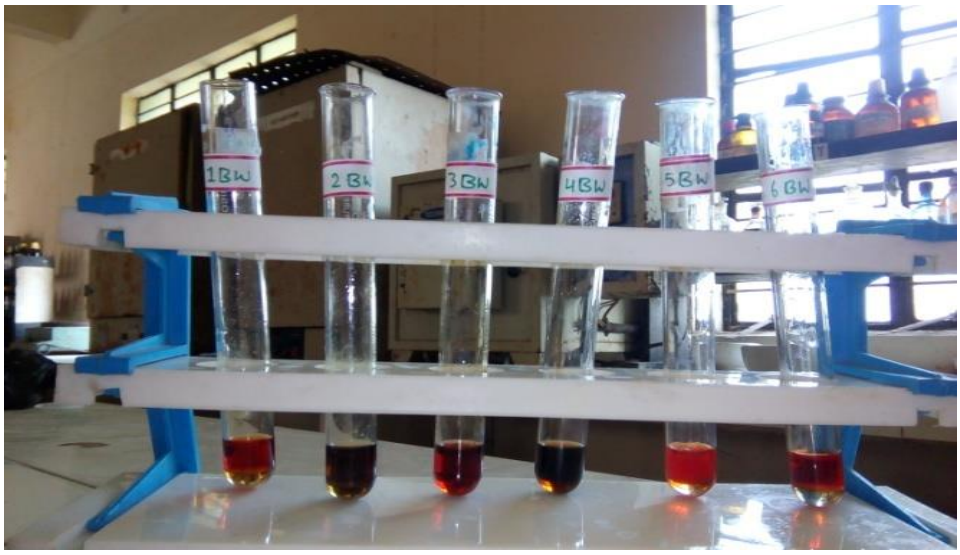

Figure-6: Identification tests for Brucine in alcohol (BA)

It may be used to differentiate Brucine from Strychnine. The additional functional group (dimethoxy -OCH_3) present in Brucine may be accountable for this difference [2]. Brucine in distilled water and alcohol developed almost similar colors when treated with different oxidizing agents and acids combination such as 1%w/v Potassium dichromate in ortho phosphoric acid/lactic acid, 0.1N Potassium permanganate in ortho phosphoric acid/lactic acid, 0.1NCeric ammonium sulphate in ortho phosphoric acid/lactic acid. So, brucine can be identified with the developed tests either by using distilled water or ethyl alcohol, as solvent used to dissolve the alkaloid has no influence on color formation in the developed tests. Brucine is a dimethoxy derivative of Strychnine. Brucine is readily oxidized by dilute Nitric acid with the formation of intense red color and can be distinguished from Strychnine [2]. May be the dimethoxy group additionally present in Brucine may be responsible for easy oxidation of Brucine and color formation. From the results of the tests it is evident that all the three alkaloids irrespective of solvent used to dissolve them produced brown color with 0.1N Potassium permanganate in lactic acid. So, this test could be used as a general test for the identification of alkaloids. Atropine sulphate and Strychnine hydrochloride could be identified with 0.1N Potassium permanganate and orthophosphoric acid either by using water or ethyl alcohol as solvent. 1%w/v Potassium dichromate in ortho phosphoric could be used as a common test for the identification of Atropine sulphate and Strychnine hydrochloride in alcohol as well as in distilled water. The same test with lactic acid in place of orthophosphoric acid could be used as a common test for identification of Atropine sulphate in water and alcohol and Strychnine hydrochloride in alcohol. The developed test using 0.1N Ceric ammonium sulphate and ortho phosphoric acid/lactic acid could be used as a common test for the identification of Atropine sulphate in alcohol and Strychnine hydrochloride in alcohol. Brucine could be identified with the developed tests either by using water or ethyl alcohol, as solvent has no influence on color formation in the developed tests.

CONCLUSION:

The identification tests developed were found to be relatively simple, economic, stable and sensitive compared to the so far reported general alkaloid tests and specific alkaloid tests for the identification of Atropine sulphate, brucine and strychnine hydrochloride. Hence, the developed identification tests could be used as alternative tests for the identification of atropine sulphate, strychnine hydrochloride and brucine.

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