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Research Article

Standardization of Marquis Reagent for the Detection of Aspirin

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Abstract

With an increasing trend of drug abuse cases encountered, better and more reliable techniques need to be established for their detection in trace amounts. Whilst color tests are applied commonly to detect and identify the drugs, Marquis Reagent test still predominantly emerges as the most effective and reliable analytical protocol.

According to literature review, the preparation of Marquis Reagent varies significantly. Different concentrations of formaldehyde solution used indicate the absence of a standardized protocol for its preparation. Hence, the objective of this research work was to standardize the Marquis reagent. This was achieved by varying different concentrations of formaldehyde solution in addition to glacial acetic acid and methanol. For this study, aspirin was used as test sample in two forms: Chemically synthesized aspirin and commercially purchased aspirin. A total of twenty procedures were employed in the study. A hundred percent solution preparation was found to be the most sensitive and reliable.

Keywords: *Marquis Reagent; Aspirin; Color Test*

Introduction

Forensic science is the field were forensic scientists are involved in the search for examination of physical traces which might be useful for establishing or excluding an association between someone suspected of committing a crime and the scene of the crime or victim. Sometimes the scientist will visit the scene itself to advice about likely sequence of events, any indicators as to who the perpetrator might be, and to join in the initial search for evidence. Other forensic scientists analyze suspected drugs of abuse, specimens from people thought to have taken them or to have been driving after drinking too much alcohol, or to have been poisoned. Others specialize in firearms, explosives, or documents whose authenticity is in doubt [1].

Forensic science is defined as "it is the application of science to the law".

It is also defined as "The application of scientific methods and techniques to matters under investigation by a court of law".

To analyze physical evidence, forensic chemistry draws on chemistry principles and concepts. Investigating the physical and chemical properties of a substance is central to forensic chemistry. Without an appreciation for these properties and the scientific method, forensic chemistry would not be possible.

Forensic toxicology refers to application toxicology to aid medico legal investigation of death and poisoning. Forensic toxicology encompasses the measurement of alcohol, drugs, and other toxic substances in biological specimens and interpretation of such results in a medico legal context. A forensic toxicologist considers the context of an investigation, in any particular physical symptoms recorded, and any evidence collected at a crime scene [2].

Forensic chemistry is the application of chemistry, in a legal setting. A Forensic Chemistry Expert assists in the identification of unknown materials found at a crime scene by applying a wide array of methods and instruments.

Along with other forensic specialists, forensic toxicologists commonly testify in court as expert witnesses regarding their findings. Forensic toxicologists follow a set of standards that have been proposed by various agencies and governing bodies, including the Scientific Working Group on the Analysis of Seized Drugs [3].

Narcotics

The term "Narcotic" is derived from the Greek word "Narkotikos", which implies the "state of lethargy or sluggishness". Narcotic drugs are those substances which give relief from pain and induce sleep but are socially unacceptable. Narcotic Drugs and Psychotropic Substances may be of natural, semi-synthetic and synthetic in origin. On ingestion, they act on the Central Nervous System to produce an altered state of mind.

According to the DFS manual procedures, the identity of a narcotic drug is to be established by at least two independent analytical parameters or till the satisfaction of the analyst. Two uncorrelated TLC systems would count as two parameters which means either the solvent system or the coating on the plate or completely different. Wherever possible, three entirely different analytical techniques may be used. For example: presumptive colour test, confirmatory crystal tests, instrumentation viz. chromatography (TLC, GLC, HPLC, etc.) and spectroscopy (IR/FTIR and UV-VIS). The actual choice of parameters is left to the sole discretion of the expert.

Different NDPS classified based on origin

According to NDPS ACT, narcotic drugs are classified into 3 categories based on their origin

1. **Natural drug:** These drugs are obtained from natural sources like plants e.g. Opium, Cannabis etc.
2. **Semi-Synthetic drug:** These drugs are manufactured by processing natural drugs with chemicals etc. e.g. Heroin, Cocaine.
3. **Synthetic drug:** These are drugs manufactured purely from synthetic substances. e.g. Mandrax, Dizapam, Barbiturates, ATS etc.

Marquis reagent

First discovered in 1896 and described by the Russian Pharmacologist, Eduard Marquis (1871 - 1944) in his dissertation in 1896 and hence, named after him. This reagent was tested for the first time at the University of Dorpat and is widely used as a simple spot-test to presumptively identify alkaloids as well as other compounds. It majorly finds application in the detection of ecstasy kits, opiates and phenethylamines [4,5].

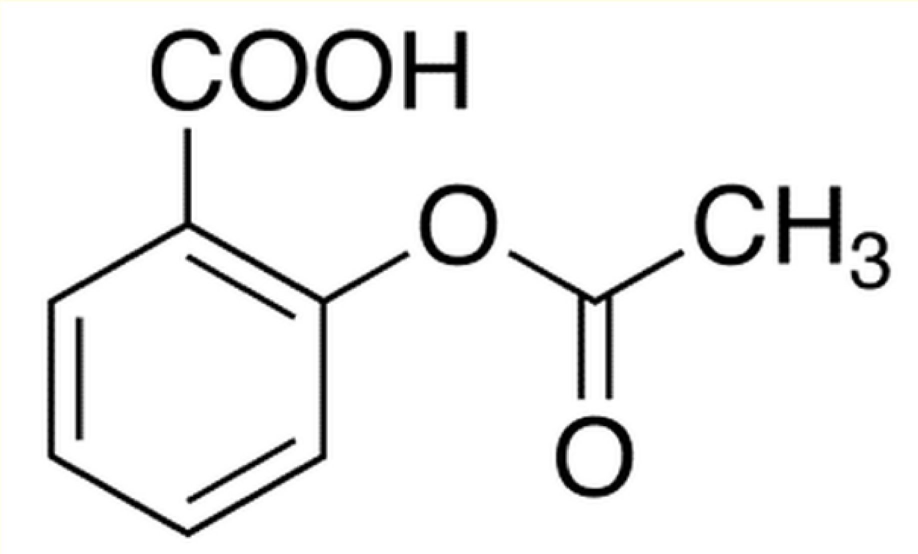
Marquis reagent is composed of a mixture of formaldehyde and concentrated sulfuric acid, which is added to the substance under analysis. The reagent has a shelf life of 12 months when stored in a refrigerator at 4°C, after which it was observed to give very slow and sometimes, incorrect colour changes.

The test is performed by scraping off a small amount of the substance into the well of a spotted porcelain tile and adding a drop of the clear and colorless reagent. The results are analyzed by observing the color of the resulting mixture, and by the time taken for the change in color to become apparent.

Acetylsalicylic acid (ASA)

Aspirin, or acetylsalicylic acid (ASA), is a common drug that is generally used as an analgesic for minor aches and pains, to reduce fever, and an anti-inflammatory drug.

Structure of aspirin



http://www.medicalisotopes.com/structures/6211.png

Mode of action

Aspirin can affect many of the major organ systems, causing significant damage. At the cellular level, it interrupts the normal cellular metabolism, causing cell death. Thus, it dramatically upsets the body's chemical and acid-base balance. The central nervous system is targeted, along with the heart, lungs, liver and kidneys. Massive overdoses can progress rapidly through the stages of respiratory failure, heart failure, kidney failure, coma and seizures leading to death.

Fatal dose: 25 to 30 gm.

Treatment

1. Urinary alkalinization is effective in enhancing urinary excretion of salicylate, although often difficult to achieve in dehydrated or critically ill patients. a) Add 100 mEq of sodium bicarbonate to 1L of D5-1/2 NS, and infuse intravenously at 200 ml/hour (3 - 4 ml/kg/hour). If the patient is dehydrated, start with a bolus of 10 - 20 ml/kg. Fluid and bicarbonate administration is potentially dangerous in patients at high risk for pulmonary edema (e.g. chronic intoxication).
2. Hemodialysis is the most effective means of removing salicylate from the body. Hemodialysis is also effective in correcting acid-base and fluid abnormalities caused by salicylate intoxication.
3. Hemoperfusion is also very effective but does not correct acid-base or fluid disturbances.
4. Multiple-dose activated charcoal (25 to 50 gm AC every 3 to 5 hours) therapy effectively reduces the serum salicylate half-life (principle of gastrointestinal dialysis), but it is not as effective as Hemodialysis. Frequent stooling may contribute to dehydration and electrolyte disturbances. Multiple-dose activated charcoal therapy also places the patient at risk for developing bowel obstruction or charcoal ileus. Cathartics should be used judiciously and may be given with every second or third dose of activated charcoal.

Materials and Methods

Materials and equipments: Test tubes, droppers, spatula, test tube stands, aluminium foil, spot tile, water bath.

Chemicals: Concentrated sulphuric acid, glacial acetic acid, salicylic acid, formaldehyde solution, methanol, distilled water, aspirin samples: Sample A: Chemically synthesized aspirin; Sample 1: Ecospirin-75 (USV private limited-dose (75 mg).

Synthesis of aspirin

Principle

Salicylic acid when reacts with glacial acetic acid and concentrated sulphuric acid when warmed in 50ºC, a product of acetyl salicylic acid is obtained.

Procedure

Preparation of acetyl salicylic acid (Aspirin) from salicylic acid

- Take 1 gram of salicylic acid in a dry conical flask.
- Add 3 ml of glacial acetic acid and 5 drops of conc. sulphuric acid.
- Heat on a water bath for 50ºC for 5 Minutes and cool
- Pour the mixture into 100 ml cold water.
- Filter-wash-dry and store it in a container.

Preparation of marquis reagent

Principle

Marquis test is one of the most common reactions for preliminary testing of suspected substances. It is used in analyzing the drug but also to identify the alkaloids or commonly used drugs, which significantly reduces its selectivity. However, its relative simplicity and especially the combination with additional tests can lead to confirmation of suspected drug in the sample. It is used primarily when testing narcotics and psychotropic substances. The reagent is used as a mixture of concentrated sulfuric acid and formaldehyde. The reaction mechanism is quite complex, it can be said that it is based on the polymerization of molecules of the test compound and formaldehyde in the acidic environment where charged oxonium or carbonium compounds are formed. Marquis reagent when used with acetylsalicylic acid (aspirin) it provides a red or orange product in about 2 - 3 minutes.

Preparation of marquis reagent

Method 1

1. 1 ml of 10% Formaldehyde in 10 ml concentrated sulphuric acid - M_1
2. 1 ml of 20% Formaldehyde in 10 ml concentrated sulphuric acid - M_2
3. 1 ml of 30% Formaldehyde in 10 ml concentrated sulphuric acid - M_3
4. 1 ml of 40% Formaldehyde in 10 ml concentrated sulphuric acid - M_4
5. 1 ml of 50% Formaldehyde in 10 ml concentrated sulphuric acid - M_5
6. 1 ml of 60% Formaldehyde in 10 ml concentrated sulphuric acid - M_6
7. 1 ml of 70% Formaldehyde in 10 ml concentrated sulphuric acid - M_7
8. 1 ml of 80% Formaldehyde in 10 ml concentrated sulphuric acid - M_8
9. 1 ml of 90% Formaldehyde in 10 ml concentrated sulphuric acid - M_9
10. 1 ml of 100% Formaldehyde in 10 ml concentrated sulphuric acid - M_{10}

Method 2

1. 10% formaldehyde solution in 10 ml of glacial acetic acid, from this solution 1 ml is taken and added to 10 ml concentrated sulphuric acid - MG_1
2. 20% formaldehyde solution in 10 ml of glacial acetic acid, from this solution 1 ml is taken and added to 10 ml concentrated sulphuric acid - MG_2
3. 30% formaldehyde solution in 10 ml of glacial acetic acid, from this solution 1 ml is taken and added to 10 ml concentrated sulphuric acid - MG_3
4. 40% formaldehyde solution in 10 ml of glacial acetic acid, from this solution 1 ml is taken and added to 10 ml concentrated sulphuric acid - MG_4
5. 50% formaldehyde solution in 10 ml of glacial acetic acid, from this solution 1 ml is taken and added to 10 ml concentrated sulphuric acid - MG_5
6. 60% formaldehyde solution in 10 ml of glacial acetic acid, from this solution 1 ml is taken and added to 10 ml concentrated sulphuric acid - MG_6
7. 70% formaldehyde solution in 10 ml of glacial acetic acid, from this solution 1 ml is taken and added to 10 ml concentrated sulphuric acid - MG_7
8. 80% formaldehyde solution in 10 ml of glacial acetic acid, from this solution 1 ml is taken and added to 10 ml concentrated sulphuric acid - MG_8
9. 90% formaldehyde solution in 10 ml of glacial acetic acid, from this solution 1 ml is taken and added to 10 ml concentrated sulphuric acid - MG_9
10. 100% formaldehyde solution in 10 ml of glacial acetic acid, from this solution 1 ml is taken and added to 10 ml concentrated sulphuric acid - MG_{10}

Procedure

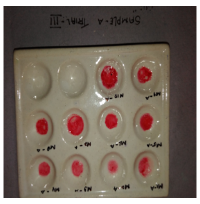
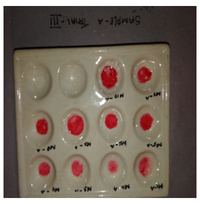
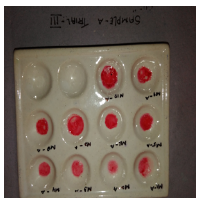
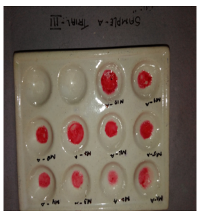
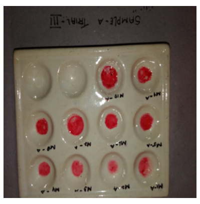
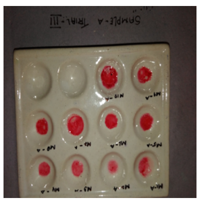
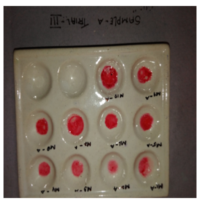
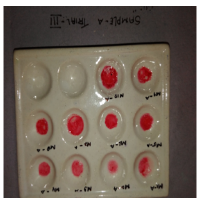
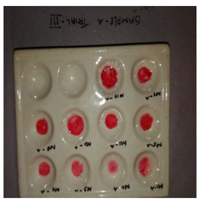
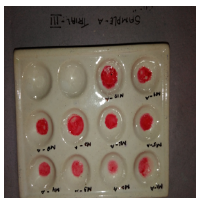
- Marquis reagent was prepared in different concentration.
- Small amount of sample (aspirin) was taken in on to a spot tile.
- 1 - 2 drops of marquis reagent was added to the sample.
- Change in colour was observed and recorded.
- 1 - 2 drops of methanol was added.
- Change in colour was observed and recorded.
- The same experiment was repeated three times for sample A and sample 1
    - Sample A: Chemically synthesized aspirin.
    - Sample 1: Purchased from the market.
- The experiment was repeated for three times and the average was taken.

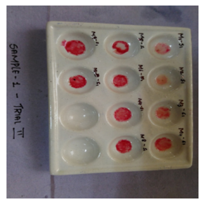
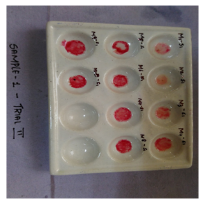
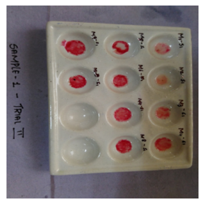
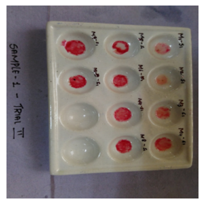
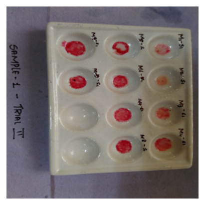
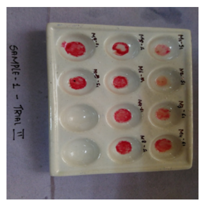
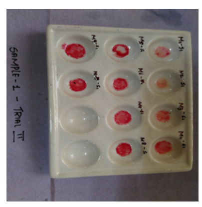
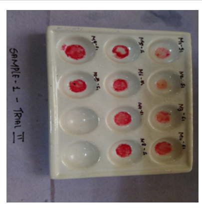
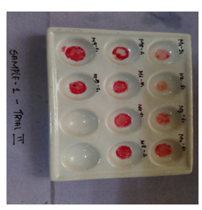
Observations and Analysis

Key

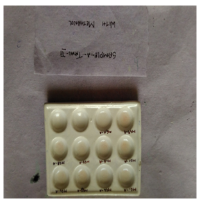
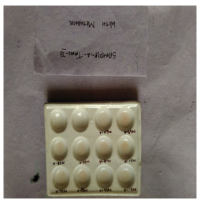
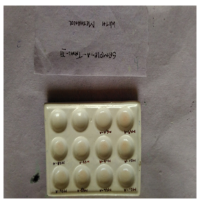
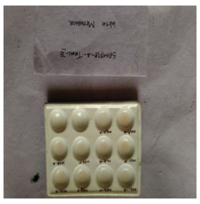
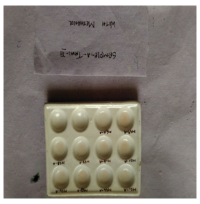
- **Color code:** a: Light Pink; b: Medium Pink; c: Dark Pink to Red.
- **Sensitivity code:** *: Color Change; **: Slight Color Change; ***: Constant Color.
- **Change after addition of methanol:** (+): Color Change; (-) = No Color Change.

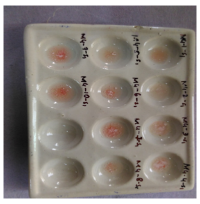
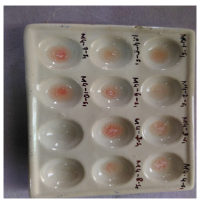
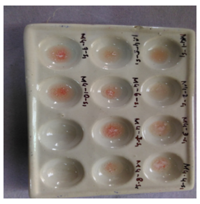
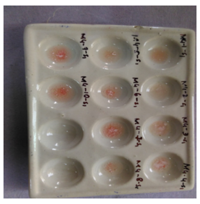
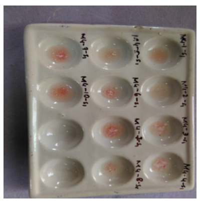
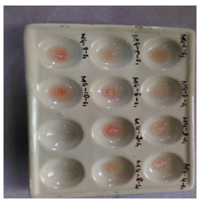
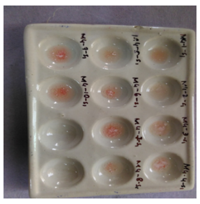
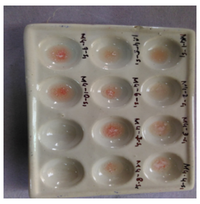
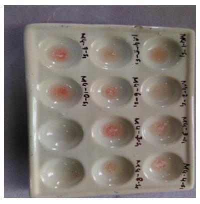
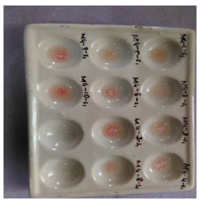
Method 1

Sample A	Color	Time of color formation in seconds	Change after addition of Methanol	Sensitivity	Image
M_1	a	Instant	+	*	
M_2	A	Instant	+	*	
M_3	A	Instant	+	*	
M_4	A	Instant	+	**	
M_5	B	10-12	-	**	
M_6	B	10 -12	-	**	
M_7	B	10 -12	-	**	
M_8	B	10 -12	-	***	
M_9	B	10 -12	-	***	
M_{10}	C	15 - 20	-	***	

Sample 1	Color	Time of color formation in seconds	Change after addition of Methanol	Sensitivity	Image
M_1	a	Instant	+	*	
M_2	a	Instant	+	*	
M_3	a	Instant	+	**	
M_4	a	Instant	+	**	
M_5	a	5 - 8	-	***	
M_6	a	10 - 12	-	***	
M_7	b	10 -12	-	***	
M_8	b	10 -12	-	***	
M_9	b	10 -12	-	***	
M_{10}	c	15 -20	-	***	

Method 2

Sample A	Color	Time of color formation in seconds	Change after addition of Methanol	Sensitivity	Image
MG_1	a	50 - 60	+	*	
MG_2	a	50 - 60	+	*	
MG_3	a	50 - 60	+	*	
MG_4	a	50 - 60	+	*	
MG_5	a	50 - 60	+	*	
MG_6	a	50 - 60	+	*	
MG_7	a	50 - 60	+	*	
MG_8	a	50 - 60	+	*	
MG_9	a	50 - 60	+	*	
MG_{10}	a	50 - 60	+	*	

Sample 1	Color	Time of color formation in seconds	Change after addition of Methanol	Sensitivity	Image
MG_1	a	20 - 25	+	*	
MG_2	a	20 - 25	+	*	
MG_3	a	20 - 25	+	*	
MG_4	a	20 - 25	+	*	
MG_5	a	20 - 25	+	*	
MG_6	a	20 - 25	+	*	
MG_7	a	20 - 25	+	*	
MG_8	a	20 - 25	+	*	
MG_9	a	20 - 25	+	*	
MG_{10}	a	20 - 25	+	*	

Results

Aspirin when treated with Marquis Reagent gave a pink to red color. The sensitivity of the color was tested through the addition of methanol. From the observations, it can be interpreted that sample A in method 1 showed light pink color instantaneously for M_1, M_2, M_3 and M_4 respectively which turned colorless on addition of methanol. A pink color developed within 10 to 12 seconds for M_5, M_6, M_7, M_8 and M_9 respectively with color remaining constant after addition of methanol. In M_{10} it showed dark reddish pink color within 15 to 20 seconds and there was no change in color after the addition of methanol. However, sample 1 for the method 1 showed pinkish yellow color instantaneously for M_1, M_2, M_3 and M_4 respectively and was rendered colorless on adding methanol, whereas a pink color was seen within 5 to 8 seconds for M_5 with no color change after the addition of methanol. A pink color within 10 to 12 seconds was observed in M_6, M_7, M_8 and M_9 respectively while M_{10} showed a dark reddish pink color within 15 to 20 seconds. The color in these samples remained constant after adding methanol.

In method 2, sample A showed light pinkish yellow color within 50 to 60 seconds for MG_1, MG_2, MG_3, MG_4, MG_5, MG_6, MG_7, MG_8, MG_9 and MG_{10} respectively with progressing color change after addition of methanol. For the sample 1, a light pinkish yellow color developed within 20 to 25 seconds for MG_1, MG_2, MG_3, MG_4, MG_5, MG_6, MG_7, MG_8, MG_9 and MG_{10} respectively with similar color changes as observed in sample A after addition of methanol.

Discussion

From the results for method 1, it can be deduced that the concentration of formaldehyde in the reagent is directly proportional to the intensity of color formation (pale pink yellow to dark reddish pink), and the time taken for development of color (instantaneous to 20seconds). Methanol addition to the sample elicited a direct relationship existed between color change (colorless to no change in color) and concentration of the reagent.

For the method 2, we can infer that the concentration of formaldehyde in the reagent is directly proportional to the intensity of color formation (pale pink yellow to pink), and the time taken for development of color (5 to 40 seconds). On addition of methanol, concentration is directly proportional to the color change in sample with a lesser intensity as compared to method 1.

From the above interpretation, we can state that method 1 was more effective, sensitive and reliable when compared to method 2, in the preparation of Marquis Reagent. Therefore, one ml of hundred percent formaldehyde solution in ten ml concentrated sulphuric acid is the best procedure for analysis as per our study [6-11].

Conclusion

Following the analysis of Marquis Reagent test by varying concentrations of formaldehyde solution, we can hereby conclude that 1ml of 100% formaldehyde solution can be used in 10ml concentrated sulphuric acid to prepare the reagent to give more reliable, sensitive and effective results.

Scope for Further Study

Chemical reactions and its kinetics vary between molecules with different structures. For better understanding the chemistry, it is important to further study Marquis Reagent test with a variety of compounds in different concentrations of formaldehyde which will enable the analyst to make a general trend prediction. Also, a better method may be developed to analyze drugs in the liquid state.

Limitations of the Study

1. Various forms of drugs were not available legally in the market hence only one type of sample was studied.
2. Aspirin was synthesized chemically and analyzed in an unpurified form due to unavailability of resources.

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