An Analysis of Froehde's and Duquenois-Levine Colorimetric Tests

By

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ABSTRACT

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Colorimetric testing is frequently used to identify unknown substances in the field. These tests are composed of a solution which changes colors in the presence of certain drugs. Each solution can yield a variety of colors to test compounds, but the tests are nonspecific. The speed of colorimetric analysis has made it ideal for onsite preliminary drug tests. In particular, the Froehde's solution can identify various opiates and hallucinogens; and the Duquenois-Levine test can identify cannabinoids. Our analysis revealed that the Froehde's test yields results beyond what is stated in the literature, but that these results are not very specific. The Duquenois-Levine test yields a number of different results, but its criteria for a positive result keep it a more specific test.

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Introduction

Colorimetric testing is the practice of combining unknown substances with particular sets of reagents to produce visibly colored products. The colors produced by each set of reagents are particular to certain types of substances. Therefore, based on the tests chosen and the colors produced, one can discern the identity of the unknown compound. These tests are useful for many reasons. First, they work with small samples of unknown compound and require only a few drops of reagent. The tests are quick, yielding results in never more than a few minutes. And thirdly, the reagents are inexpensive on a per use basis. For these reasons, colorimetric tests are used by the police for on-site drug analysis. However, the ability of these tests is limited by their accuracy. Specifically, for every test, there are a number of compounds unrelated to the target drug which can produce the same color. These results are known as false positives. Because of the errors induced by false positives, police investigations use colorimetric tests as a form of presumptive analysis. This way, the results are used to determine whether or not an unknown substance should be taken back to the lab for a more in-depth analysis. Such analyses include but are not limited to gas chromatography mass spectroscopy (GC-MS), and high performance liquid chromatography (HPLC).

In this experiment, we examined two particular colorimetric tests, the Froehde's test and the Duquenois-Levine test. The Froehde's test is composed of a mixture of sodium molybdate dissolved into sulfuric acid. It reacts with aromatic compounds that are susceptible to oxidation, reduction, and substitution, and is a known identifier of various hallucinogens and opiates such as LSD and codeine (Butler 1966, O'Neal, 2000).

The Duquenois-Levine test reacts to the active ingredient in marijuana, tetrahydrocannabinol (THC) and is composed of two tests: the Duquenois test, and the Levine modification (Smith, 2004). The initial test begins with an extraction of the sample with petroleum ether followed by an addition of the Duquenois reagent, composed of acetaldehyde and vanillin. After the extract and reagent have mixed, hydrochloric acid (HCl) is added and the color recorded (Duquenois, 1938). However, by the 1960s, follow up studies had displayed that the test was not specific for THC by demonstrating that various other oils and cannabinoid compounds could trigger positive responses (Kelly 2012). In response, the Levine modification alters the procedure by adding chloroform after the colors have developed from the hydrochloric acid. The two solutions do not mix, resulting in two layers, and the colors of each are read.

The goal of this experiment will be to analyze what reactions these compounds can form against various different kinds of drugs. To simulate other drugs, we're using a specific set of compounds which act as analogs of the target drugs of various other tests. Using these analogs allows us to run additional tests at lower costs and without the risks of handling illegal or dangerous chemicals. By examining how these test reagents react towards compounds both like and unlike their target molecule, we can find new ways for these tests to identify compounds.

Materials and Methods

The drugs used in this analysis are as follows: acetaminophen, chloroacetophenone, sodium glycolate, indole, methapyrilene, pseudoephedrine, quinine, chlorpromazine, eugenol, eucalyptus oil, and cypress oil. For each of the tests below, these compounds were examined for what colors they produced and how they relate to the literature.

Froehde's Test:

The reagent solution was prepared according to the National Institute of Justice's standards, but scaled down by a factor of four to avoid creating a vast excess of solution. Therefore, 0.125 grams of sodium molybdate was mixed with 25mL of hot concentrated sulfuric acid. The solution was then stored and capped in a 40mL glass cylinder. Using a ceramic twelve well plate, roughly 0.5 - 1 grams of a selected solid drug or 250μL of a liquid drug was placed into a well and then a measured amount of test solution was added. Each drug was tested with various quantities of solution in order to examine the range of colors produced and record any changes that took place. Photos of each test were taken, as well as notes on the colors and reactions. Production of a color was considered a positive response, and the lack of color was a negative response.

Duquenois-Levine Test:

The reagent solution was prepared according to the National Institute of Justice's standards but scaled down by a factor of four to conserve resources. Using a ceramic twelve well plate, roughly 0.5 to 1.0 grams of a selected drug was added to a well. The drug was then treated with Duquenois reagent solution, followed by hydrochloric acid, and then chloroform. The mixture would be mixed by a metal spatula after each addition of a solution. Photos and notes were taken at two points: after the addition of HCl, and after the addition of chloroform. With the addition of chloroform, the lighter acid layer would sit on top and be visible as an outer ring in the well, and the heavier chloroform layer would be seen in the center, resting at the base of the well. In each test, the colors produced after the addition of HCl, and in the two layers after the addition of chloroform, were noted. Although all produced colors were recorded in this analysis, a positive result for THC is documented as a production of a violet color with the addition of HCl that transfers to the chloroform layer (Kelly 2012). The transfer of a blue color to the chloroform layer is also acceptable.

In addition to running the test in twelve well plates, each Duquenois-Levine test was run once in a test tube to get a better view of the distribution of colors between the acidic and chloroform layers.

Results and Discussion

Table 1. Results of Froehde’s and Duquenois-Levine Tests

Drug	Froehde's	Duquenois Levine	
		Acid	Chloroform
Acetaminophen	Blue	No reaction	Clear
Chloroacetophenone	Yellow	No reaction	Clear
Glycolate	No reaction	No reaction	Clear
Indole	Red --> Yellow	Red	Red
Methapyrilene	Dark Brown	Blue/Pink	Clear
Psuedoephedrine	No reaction	beige	Clear
Quinine	Pale Yellow	Yellow	Clear
Chlropromazine	Dark Pink	Pink	Clear
Eugenol	Very Dark Purple	Moss Green	Dark Yellow Green
Eucalyptus	Brown	Purple	Clear
Cypress	Reddish Brown	Dark Purple	Clear

Although many of these results match the literature (table 3, appendix), some of them do not which will be discussed at each individual analysis.

Froehde’s Test:

Figure 1. Froehde’s Test results for Acetaminophen, chloroacetophenone, glycolate, and pseudoephedrine

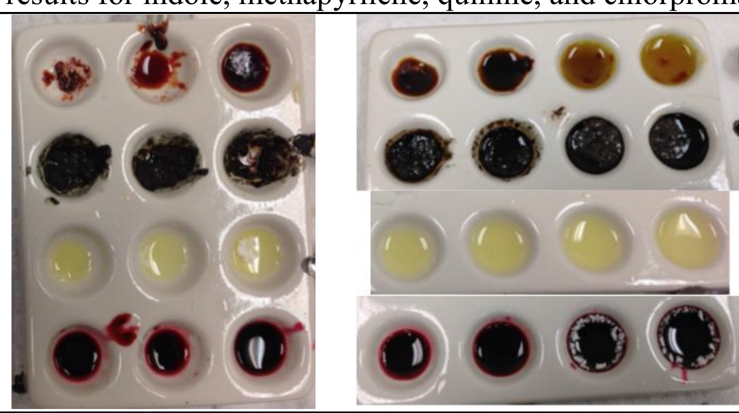
Acetominophan	
Chloroacetophenone	
Glycolate	
Pseudoephedrine	

Drug	Quantity of Solution (µL)						
Acetominophan	250	500	750	50	100	200	250
Chloroacetophenone	250	250	150	50	100	200	250
Glycolate	150	150	150	NA			
Pseudoephedrine	150	150	150				

Here it is evident that acetaminophen yields a bright blue color at most concentrations. But when low quantities of Froehde's reagent are added, it displays a mixture of both blue and yellow. And at very low quantities it is yellow. Interestingly, a blue color isn't reported in the literature for any compound reacting to Froehde's test. These two properties make this reaction very unique.

Chloroacetophenone yields a yellow color at every concentration, varying in intensity in proportion to how much reagent is added. According to the literature, it is similar in color to oxycodone (Table 3). Neither glycolate nor pseudoephedrine yielded any results. This is likely due to the visible insolubility between the compounds and the solution.

Figure 2. Froehde's test results for indole, methapyrilene, quinine, and chlorpromazine

Indole	
Methapyriline	
Quinine	
Chlorpromazine	

Drug	**Quantity of Solution (μL)**						
Indole	20	50	100	100	200	400	600
Methapyriline	100	75	50	100	200	400	600
Quinine	100	150	200	200	400	600	800
Chlorpromazine	100	50	200	100	200	400	600

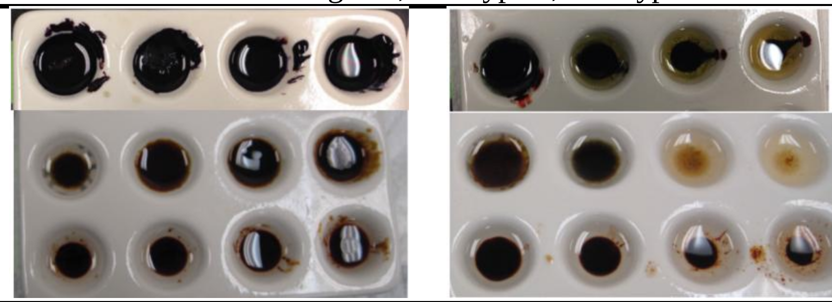
Indole reacted very strongly giving a dark red color when mixed with lower quantities of solution. However, when the proportion of indole to Froehde's solution shifted away from indole, the color became a clear dark yellow. In either case, over time the solution became brown, meaning that results must be interpreted quickly. As a reddish brown, the color most closely associates with the result for doxepin in the literature (Table 3, appendix).

At lower concentrations, methapyrilene had a dark brown color that became a dark green as time passed. It has a very thick, nearly solid consistency. At higher concentrations, it simply appears dark brown to the point of black. It also has a more liquid composition, but it is heterogeneous in form and appears as a solid sitting inside of a liquid solution. Both components are very dark in color and not transparent. This color could be associated with results from codeine or contac.

Quinine produces a transparent yellow color when the Froehde's reagent is added, and does not appear to change much with increased additions of reagent. Although its color is similar to chloroacetophenone, it is lighter than it, and varies less than it does. This conflicts with the literature which labels quinine as non-reactive.

Chlorpromazine appears black in the center, but has a dark pink ring around the edge which implies that its actual color result is dark pink. However, at higher concentrations, it is more difficult to distinguish this and it appears black. This correlates with the literature's listing of deep red for chlorpromazine.

Figure 3. Froehde’s test results for eugenol, eucalyptus, and cypress

Eugenol								
Eucalyptus								
Cypress								
Drug	**Quantity of Solution (µL)**							
Eugenol	250	100	50	25	100	50	30	20
Eucalyptus	50	100	150	200	100	50	30	20
Cypress	50	100	150	200	100	50	30	20
	250 µL sample				**500 µL sample**			

The Eugenol produces a very powerful reaction with the Froehde’s solution to the point of combustion from the exothermic reaction. To compensate, the amount of eugenol was doubled and decreased amounts Froehde’s reagent were used to widen the ratio of drug to solution. By the point of 50/500, it appears that the reaction had surpassed its limit and there was excess eugenol. At all times the reacted portion always looked black in the well; however upon cleaning, the solution actually smeared purple which may indicate the actual color of this test. Although deep purplish red is listed for several compounds in the literature, there’s nothing yet listed for deep purple.

Eucalyptus and cypress also reacted very exothermically, but not to the same degree as the eugenol. They each stained dark red-orange, although the eucalyptus appeared brownish yellow at extremely low treatments of Froehde’s reagent. This color is not yet listed in the literature.

Duquenois-Levine Test:

Table 2. Test reagent quantities

Solution	Number of Drops							
Duquenois	1	2	3	4	5	6	7	8
HCl	1	2	3	4	5	6	7	8
Chloroform	3	6	9	12	15	18	21	24

Figure 4. Results for Eugenol, Eucalyptus, and Cypress after addition of HCl

Eugenol

Eucalyptus

Cypress

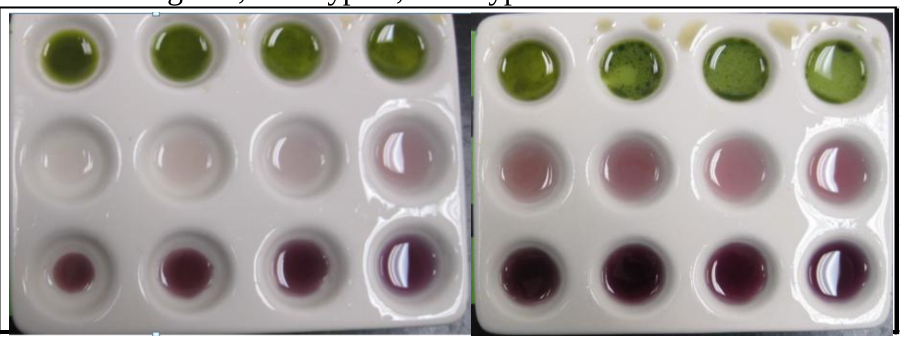
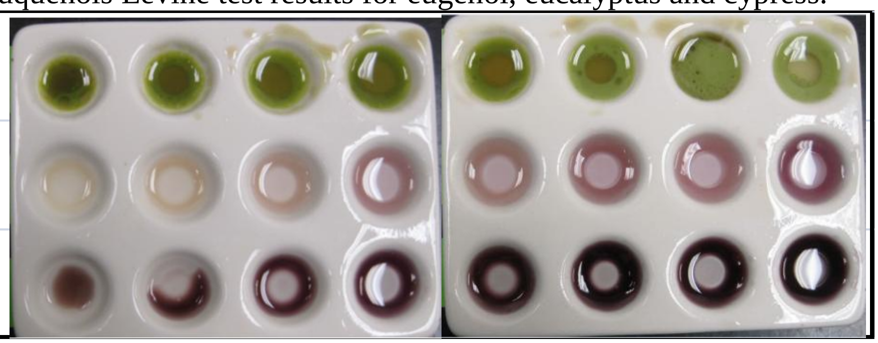


Figure 5. Duquenois Levine test results for eugenol, eucalyptus and cypress.

Eugenol

Eucalyptus

Cypress



The eugenol reacted strongly, regardless of how little test solution was added to it. In all cases it gave off a cloudy green color in the acidic layer, and a clear but dark yellow green color. This isn’t visible in every well, but is made most visible in the fifth well.

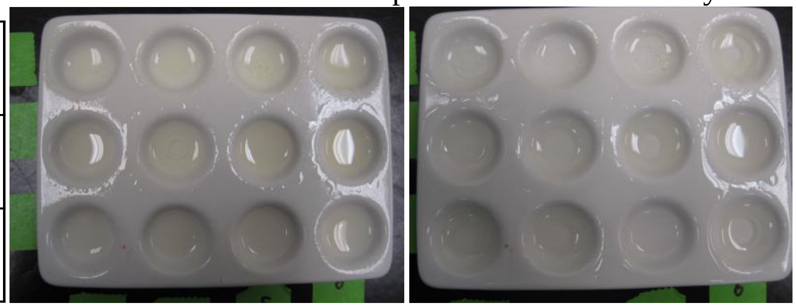
The eucalyptus is difficult to distinguish at lower quantities of test solution, but at higher quantities, it displays a distinct pink in the acid layer and darkens to a nearly purple color at the highest concentration. In the chloroform layer, it is difficult to determine if there has been a color change. If so, it appears to be a shade of purple which would indicate a positive result for THC.

The Cypress gives a strong reaction to the hydrochloric acid at all levels, but shows change in the presence of the chloroform. In the acid layer, there is a dark purple color. In the chloroform layer, it appears to be clear.

All three of these compounds were theorized to yield a positive test result (Kelly 2012). In fact, they each gave a positive result for a Duquenois test, but yielded negative results with the Levine modification. This illustrates both the error with the previous test and the specificity of the new version. In addition, these results allowed for a specialization in the other tests. The best results appeared at the greatest proportions of test solution to drug. However, at the highest proportions, the solutions were at the brink of overflowing out of their wells. Therefore, the middle four proportions from well three until well six were picked for examination of the other drugs.

Figure 6. Duquenois-LevineTest results for Chloracetophenone and Sodium Glycolate

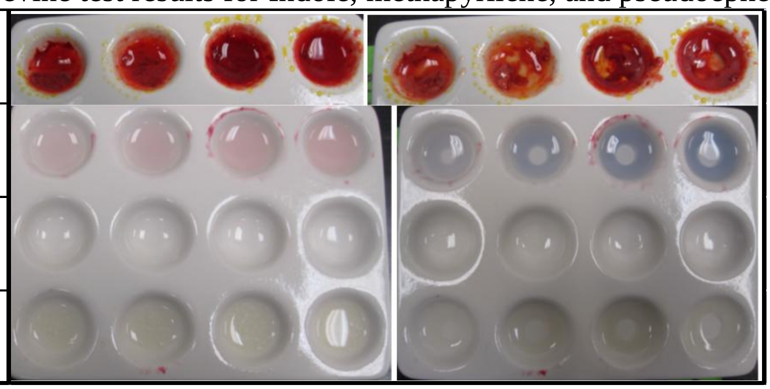
Acetominophan
Chloroaceteophenone
Sodium Glycolate



In each of these solutions, there is no color formation. On the other hand, acetominophan and chloroacetophenone did actualy display a reaction visible in the rapid movement of certain particles in the solution. But because this was not color forming, the drugs' reactions to the Duquenois-Levine test is negative.

Figure 7. Duquenois-Levine test results for Indole, methapyrilene, and pseudoephedrine

Indole
Methapyrilene
Pseudoephedrine



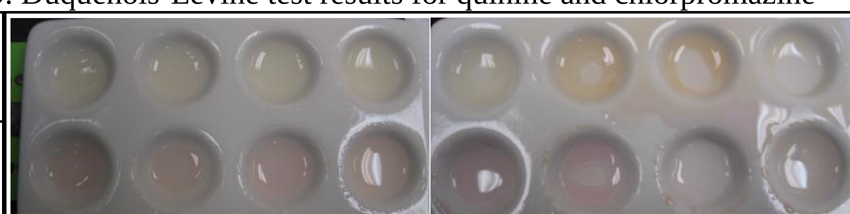
When hydrochloric acid was applied to the indole, there was an instant reaction resulting in a red solid and very red solution. Although a color was produced, red is not within the range of colors for a positive result in this test and the formation of solids is also incorrect and it was impossible to detect any layering with the addition of chloroform. However, it is a very distinctive result which could be useful for identifying this compound among others.

Addition of the hydrochloric acid to the methapyrilene resulted in a pink solution. Surprisingly, this became a light but distinct blue with the addition of the chloroform. What is not seen is that the color was initially much lighter, and slowly darkened to the shade seen in the photo above over the course of a few minutes. This color remained in the acid layer however, and there is little no visible color in the chloroform layer. Furthermore, additional testing revealed that if an excessively large sample of methapyrilene is used, the solution will stay pink, although it will lighten over time. These results are useful for the identification of this particular compound using this test.

Figure 8. Alternate response for methapyrilene

The pseudoephedrine did not yield a color change, however it does dissolve when the ratio of solute to solvent was low. Upon dissolution, it produced a very pale beige color. However, this is nearly identical to a control, and therefore is not valid.

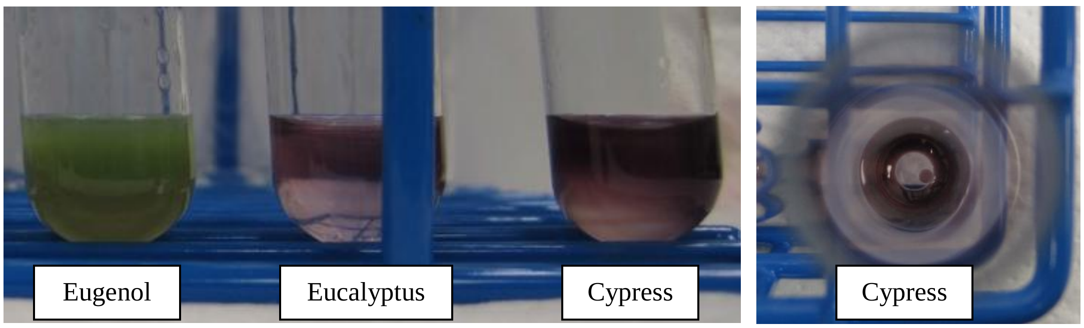
Figure 9. Duquenois-Levine test results for quinine and chlorpromazine

Quinine	
Chlorpromazine	

Quinine does react with the test reagents and displays a light but visible yellow color. It's not the correct color for a positive result, but its color production is useful for identification of this compound. The color is only visible in the acid layer, and the chloroform layer appears to be clear.

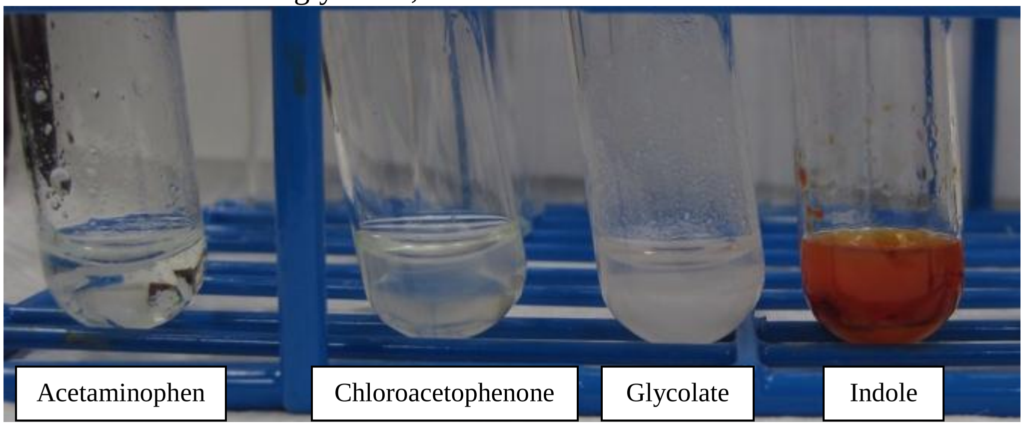
The chlorpromazine gives off a pink color in the acid layer and is clear in the chloroform layer. For both of these tests, the images may give the impression that the color began to fade. Unfortunately this is actually because at the higher concentrations, a reaction began to take place that consumed the acid layer.

Figure 10. Duquenois-Levine test results for eugenol, eucalyptus, and cypress in test tubes



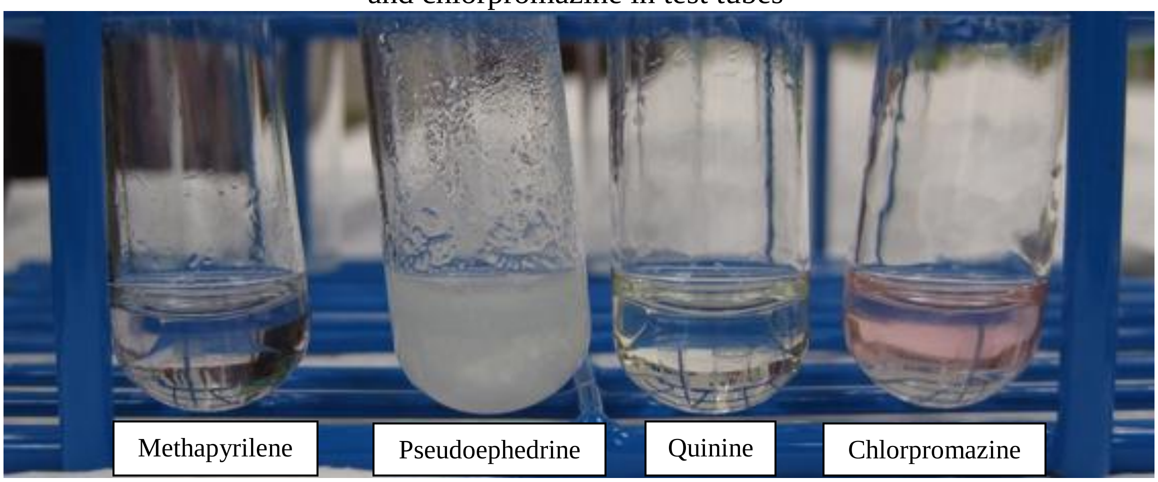
Using the test tube, it is much easier to notice the separation of layers. The two shades of green are seen against one another. In the eucalyptus oil tube, it becomes evident that some of the purple from the acidic layer does transfer over to the chloroform layer yielding a false positive result for THC. The cypress sample appears to do the same and to greater degree; however, the chloroform layer is much clearer than it appears, it is just surrounded by the acid layer. This can be seen through a top down look at the vial.

Figure 11. Duquenois-Levine test results for acetaminophen, chloroacetophenone, glycolate, and indole in test tubes.



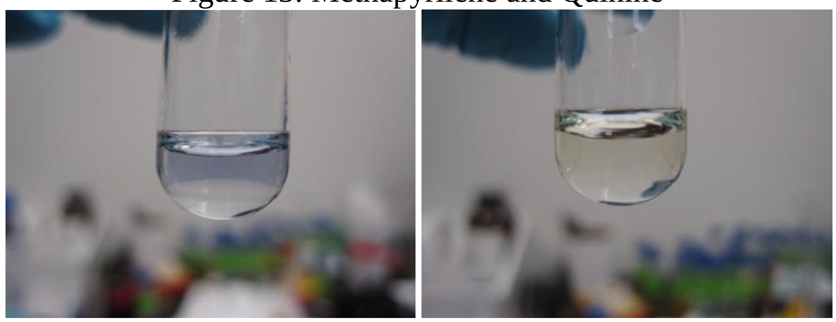
Similar to the trays, acetaminophen, chloroacetophenone and glycolate do not give a colored response. Indole has formed a red solution with solid pieces in it.

Figure 12. Duquenois-Levine test results for methapyrilene, pseudoephedrine, quinine, and chlorpromazine in test tubes



Methapyrilene shows a very faint layer which developed more with time. A later photo of the same sample is seen in figure 13. The pseudoephedrine did not react, although a layer is vaguely visible. Although quinine appears to have lost its layers; in reality the chloroform layer is inside and the acidic layer is on the outside. Like the methapyrilene, this developed over time and is depicted in figure 13. It appears completely clear in the middle, therefore the chloroform layer is clear. Chlorpromazine on the other hand has a very distinct separation of layers with a pink acid layer and no color in the chloroform layer.

Figure 13: Methapyrilene and Quinine



On one hand, the Duquenois-Levine has proven to be surprisingly specific test for THC considering the number of articles speaking to the contrary. However this only holds true if you constrain the criteria to a purple color in the acid layer followed by a purple or blue color in the chloroform layer. Unfortunately various sources have different criteria for a positive result, some being as vague as declaring a purple color to confirm the presence of marijuana. Under that condition, the test is not very specific for THC and rather problematic. There were a number of other characteristics that appeared in our analysis of the Duquenois-Levine test which can be used as a means of identifying different chemicals based not on whether they meet the criteria of THC, but based on how they react to the solution.

Colorimetric testing is an invaluable resource for onsite drug analysis. The tests are simple to run, the results are rapid, and they can rule out many compounds. However, if used alone, they have limited functionality. Our analysis of Froehde's reagent and the Duquenois-Levine test displayed that each test can display many results, but not results are specific to one drug. Several compounds under the Froehde's test were various shades of red or purple which were difficult to sort with the literature. In the Duquenois-Levine test, we found it to be very specific to THC with the Levine modification, but various compounds created yellows, pinks, or purples. To compensate, one can combine colorimetric analysis with other types of analysis such as TLC, HPLC, GCMS or any other drug analysis. However, despite the scientifically proven limits, there is record of law enforcement officers becoming increasingly dependent upon colorimetric tests for confirmatory analysis rather than presumptive analysis (Kelly 2012). In such cases, individuals can suffer terrible penalties under an incorrect judgment. If this desire to rely on colorimetric testing prevails, then a better system is required. Rather than using specified tests for specific substances, using a series of tests on all substances can allow for the results of one test to eliminate the false positives of another test. This would greatly increase the specificity of colorimetric testing, preventing a great number of false accusations.

Appendix

Table 3. List of literature values for Froehde's and Duquenois Levine tests

Literature Results	Color	
Compound	**Froehde's Test**	Duquenois Test
Aspirin	Grayish purple	NR
Acetaminophen	NR	NR
Alprazolam	NR	NR
Baking soda	NR	NR
Brompheniramine maleate	NR	NR
Chlordiazepoxide HCl	NR	NR
Chlorpromazine HCL	Very deep red	NR
Codeine	Very dark green	NR
Contac	Moderate olive brown	NR
Diacetylemorphine HCl	Deep purplish red	NR
Diazepam	NR	NR
Dimethoxy-meth HCl	Very yellow green	NR
Doxepin HCL	Deep reddish brown	NR
Dristan	Lightbluish green	NR
Ephedrine HCl	NR	NR
Excedrin	NR	NR
Hydrocodone tartrate	NR	NR
LSD	Moderate yellow green	NR
Mace	Light olive yellow	Strong Reddish Purple, very light purple
MDA HCl	Greenish black	NR
Meperidine HCl	NR	NR
Methaqualone	NR	Reaction (not listed)
Methylphenidate HCl	NR	NR
Morphine monohydrate	Deep purplish red	NR
Nutmeg	NR	Pale reddish Purple, light grey purplish red
Opium	Brownish black	NR
Oxycodone HCL	Strong yellow	NR
Phencyclidine HCl	NR	NR
Propoxyphene HCL	Dark grayish red	NR
Pseudoephedrine HCl	NR	NR
Quinine HCL	NR	NR
Salt	NR	NR
Sugar	Brilliant yellow	NR
Tea	NR	Light Yellow Green
Tobacco	NR	NR

*NR: No Reaction

Works Cited

Butler, W. P. Methods of Analysis, IRS Publication. 1966, 341, 136

Duquenois, P.; Moustaha, H.N. Identification and Assay of Cannabis Indicia. J. Egypt. Med. Assoc., 1938, 21, 21.

Fuller H. C. The Chemistry and analysis of drugs and medicines. John Wiley and Sons, Inc: New York. 1920.

Kelly J.F.; Addanki, K.; Bagasra, O. The non-specificity of Duquenois-Levine field test for marijuana. *Open Forensic Sci J* [online] 2012, 5, 4-8 http://benthamscience.com/open/toforsj/articles/V005/4TOFORSJ.pdf

O'Neal C. L.; Crouch D. J.; Fatah A. A. Validation of twelve chemical spot tests for the detection of drugs of abuse. Forensic Sci Int. 2000, 109, 189-201

Smith, F. J. Handbook of forensic drug analysis. Elsevier Academic Press: Burlington, 2004.