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Issue of False Amphetamine Field Test Positives Caused By Sugar. Use of Baeyer Test as a Secondary Test Solution.

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Abstract

The Marquis reagent is a well-established and widely used chemical presumptive test for 3,4-Methylenedioxymethamphetamine (MDMA) and methamphetamine. It is composed of concentrated sulfuric acid and 40% formaldehyde, which act upon alkaloids causing them to complex into larger molecules. This complexation causes a color change that can be visually interpreted as a positive or negative result. Almost any sugar molecule can be complexed in this way as well, due to their many OH groups. Experimentally it was found that the sugar molecules did complex with one another when the Marquis test was administered. The color produced by this reaction was brownish-red. Meaning it tested positive for amphetamine, but not methamphetamine specifically. Methamphetamine is expected to turn a light blue color, while other amphetamines are expected to turn brownish-red. A principal molecular difference between methamphetamine and common sugars is the presence of carbon-carbon double bonds. All amphetamine molecules contain three of these pi bonds, while common sugars such as sucrose, glucose, and fructose do not contain any pi bonds. The Baeyer test is a well-established organic chemistry test that is not currently used in forensic field tests. The test uses a 1% potassium permanganate solution and acetone to react with pi bonds. If pi bonds are present in the sample, an aqueous layer will appear in the violet solution. If not, the solution will be uniformly violet. This was supported as an effective method of differentiation as in our experimental tests the control samples of pseudoephedrine were consistently positive for the test. While the samples of common sugar consistently did not test positive. This implies that there is a consistent issue of sugar samples testing positive for amphetamines, and the Baeyer test could be used as an effective secondary field test to decrease the number of individuals falsely accused of possessing amphetamines.

Introduction

Methamphetamine ($C_{10}H_{15}N$) and MDMA ($C_{11}H_{15}NO_2$) are drugs that are considered controlled substances, and as a result, samples of suspected methamphetamine or MDMA are taken into police custody upon being found. The difficulty is determining if a substance is an illicit drug or not, given that they are often discovered in small amounts. If a police officer suspects that they have found an illicit drug in the car, they will most likely use a chemical field test to determine if it is the suspected drug or not. Specifically for methamphetamine, there are several different chemical field tests in use today, from the Chen-Kao test, to the Simon's test, to the gallic acid test, and the Marquis test (UNODC, 2006). Each of these methods uses a slightly different combination of chemical agents to see if the substance is methamphetamine or not.

One of the most versatile methods is the Marquis reagent. It is composed of concentrated sulfuric acid (H_2SO_4) and 40% formaldehyde (CH_2O) (Marquis Test, Oxford Reference). When the methamphetamine sample being tested comes into contact with these reagents, a complex is formed between pairs of the molecules. Each is connected to the other in the location where they formerly had their least strictly hindered hydrogen. Once the two molecules are formed together, the sulfuric acid pulls away one of the center hydrogens to complete the reaction (Supplemental pages Figures 8 and 9). The product appears as an orange-brown substance for methamphetamine, and as a purple-black substance for MDMA (Identidrug Chart, 2021).

This reaction provides clear and fast results for law enforcement officers as they are no longer able to assume that the substance in question is not a controlled substance. However, this reaction is merely a presumptive test, so the sample will be sent to a forensic laboratory to be confirmed as an illicit substance. Confirmatory testing involves a gas chromatograph and mass spectrometer (Harper et al., 2017). Depending on how much of the substance was found, the suspect may be put into temporary custody while confirmatory testing is completed. This poses a problem for false positives. If the substance in question comes up as a positive result on the field test but is not an illicit substance, then an innocent person may have to spend weeks to months of their life in custody because of a false positive.

This exact problem has allegedly occurred to several individuals as a result of a false positive from sugar crystals (NES Ink. Staff, 2018). Realistically, if an average person is to have a crystalline substance in their car at any given moment, it is likely sugar. In these cases, the sugar had been tested and seen as positive for methamphetamine. While these stories are easy to find, there has yet to be experimental research proving that this is a consistent problem. It is entirely possible that on these occasions, the tests being used were faulty. To determine if this was a genuine cause for concern or not, common sugar was tested using standard NIK field tests.

The Narcotics Identification Kit, or NIK, field tests are some of the most widely used narcotic test kits by law enforcement officers. The company makes chemical field tests for almost any controlled substance commonly found on drug offenders. Their methamphetamine/MDMA tests are composed of two parts. Test A is the Marquis test, with an ampule for formaldehyde and one for sulfuric acid. Once administered a first color change is noted. Methamphetamine for example is expected to turn an orange-brown color, and MDMA is expected to turn a purple-black color. The second part of the test is referred to as test U. The reagents that make up test U are currently listed as proprietary information. It is used as a secondary test for methamphetamine and MDMA. Methamphetamine is expected to turn light blue when this reagent is added, and MDMA is expected to turn dark blue (Identidrug Chart, 2021).

In theory, the issue with a sugar molecule is that when it reacts with sulfuric acid and formaldehyde, it can form many different complexes. This is mostly due to their large number of hydroxyl groups. In principle, all of these groups can react with the sulfuric acid, eventually leading to the addition of the formaldehyde onto the sugar molecule. From there, the molecule can react with other sugar molecules in opposingly charged states to produce a complex (Supplemental pages Figures 10 and 11).

To ensure that the Marquis tests being used in this study were not faulty, a known control that is molecularly similar to methamphetamine was used. Pseudoephedrine ($C_{10}H_{14}NO$) is a precursor molecule that is often used in methamphetamine synthesis. The only molecular difference between pseudoephedrine and methamphetamine is that pseudoephedrine contains one extra hydroxyl group (Oxford Reference, 2005). It complexes in the same way that methamphetamine does, however when the U test is administered it should turn a brownish-red instead of light blue. This result shows the molecule being tested is an amphetamine, though the extra OH group on the molecule prevents it from proving to be methamphetamine specifically. Because pseudoephedrine is an amphetamine, but not methamphetamine, it provides a check for accuracy for the Marquis test.

If sugar proved to consistently be a source of false positives, a method would need to be implemented to differentiate between sugar and methamphetamine. The Baeyer test provides a solution to this problem. It is a well-established organic chemistry test that can differentiate between molecules with and without pi bonds (Tests For Unsaturation). The test consists of 1% aqueous potassium permanganate ($KMnO_4$) combined with concentrated acetone (C_3H_6O). When a pseudoephedrine or methamphetamine molecule interacts with these reagents, the pi bonds on the molecules are broken and distributed between two of the oxygens on the potassium permanganate. This intermediary molecule draws hydrogens from the acetone to form two OH groups on the pseudoephedrine/ methamphetamine molecules and reduces the $KMnO_4$ to $KMnO_2$. The $KMnO_2$ appears as an aqueous layer in the solution (Supplemental pages Figures 12 and 13).

The Baeyer test only works on molecules that contain pi bonds, so it will not react with common sugar molecules. The sugar molecules are already saturated with hydroxyl groups and are not, therefore, capable of reducing $KMnO_4$ to $KMnO_2$. With the lack of reduction, comes the absence of the aforementioned brown aqueous layer. In the field, these two tests could be used in succession to presumptively indicate if the substance is methamphetamine or not, and then confirm that it contains pi bonds. In theory, this should eliminate the concern for false positives caused by common sugar.

Materials and Methods

2.1 *Chemicals reagents*

The potassium permanganate was purchased from Thermo Fisher Scientific. The pseudoephedrine was purified from SUDAFED® tablets. The lab-grade ethanol, lab-grade acetone, and sugar were obtained from the University of Nebraska-Lincoln forensic science laboratory. The methamphetamine/ MDMA field tests (NIK tests) were purchased from Forensics Source$_{TM}$, these contained the concentrated sulfuric acid and 40% formaldehyde required for the Marquis test.

2.2 *Pseudoephedrine extraction*

A mortar and pestle were first used to finely grind the pseudoephedrine pills into a powder form. This powder was dissolved in 200mL of warm lab-grade ethanol and then filtered through a coffee filter. The filtrate was then placed in a hot water bath in a fume hood to evaporate off the ethanol. The powder left in the bottom of the flask was dissolved in 50mL of warm distilled water and then recrystallized out of the solution using an ice bath. This crystalline pseudoephedrine product was set aside to dry for one week.

2.3 *NIK testing*

The purified pseudoephedrine and sugar were gathered in a fume hood and separated into small portions for testing. The test A reagents were broken inside of the provided pouch, and then pipetted onto the allocated samples. Next, the test U reagents were broken inside of the pouch and pipetted onto the allocated samples. After mixing with test A, color changes were noted. After mixing in test U, any additional color changes were noted.

2.4 *Baeyer testing*

First, 200mL of 1% potassium permanganate was created. The purified pseudoephedrine and sugar were then gathered in a fume hood and separated into small portions for testing. 0.5mL aliquots of acetone were then measured out into a small test tube, and the sample was added to the solution. Once dissolved, the 1% potassium permanganate was added dropwise into the solution. If an aqueous layer formed, it was marked as a positive result. If not, it was recorded as a negative result.

2.5 *NIK testing data analysis*

The NIK tests results were split into two categories, test A (Marquis test) and test U. The results for test A were placed into two categories, positive for amphetamines and negative for amphetamines. The result was interpreted using the color chart provided with the testing kit. The results for the U test were placed into categories of either negative for methamphetamine, or positive for methamphetamine. The data was collected for both pseudoephedrine and sugar for each of these tests, then analyzed using the chi-squared method. The significance value used was 0.05, with one degree of freedom for each test. Once the chi-squared value was calculated, it was used to find the p-value. This p-value provided clarity as to whether or not the results were significant.

2.6 *Baeyer testing data analysis*

The Baeyer test results were split into two columns, negative and positive test for double bonds. The data points were collected and organized into tables to be analyzed using the chi-squared method. The data was collected for both pseudoephedrine and sugar, then analyzed using the chi-squared method. The significance value used was 0.05, with one degree of freedom for both tests. Once the chi-squared value was calculated, it was used to find the p-value. This p-value provided clarity as to whether or not the results were significant.

Results

3.1 *NIK Primary Test A on Sugar*

Qualitative results of 25 tests for sugar using NIK Primary Test A showed positive results for amphetamines (Figure 1). These results were consistent with expectations. With these expectations being met the hypothesis is supported with a P-value of 1 (df=1).

3.2 *NIK Secondary Test U on Sugar*

Qualitative results of 25 tests for sugar using NIK Secondary Test U showed negative test results for methamphetamine (Figure 2). These results were inconsistent with expectations. With these expectations not being met the hypothesis is rejected in this regard with a P-value of 0.00001, and X^2 Value of 25 (df=1).

3.3 *NIK Primary Test A on Pseudoephedrine*

Qualitative results of 25 tests for pseudoephedrine using NIK Primary Test all showed positive results for amphetamines (Figure 3). These results were consistent with expectations. With these expectations being met the hypothesis was supported with a P-Value of 1 (df=1).

3.4 *NIK Secondary Test U on Pseudoephedrine*

Qualitative results of 25 tests for pseudoephedrine using NIK Secondary Test U showed negative results for methamphetamine (Figure 4). These results were consistent with expectations. With these expectations being met the hypothesis was supported with a P-Value of 1 (df=1).

3.5 *Baeyer Test on Sugar*

Qualitative results for 25 tests of Sugar using the Baeyer test showed negative results(Figure 5). These results were consistent with expectations. With these expectations being met the hypothesis was supported with a P-Value of 1 (df=1).

3.6 *Baeyer Test on Pseudoephedrine*

Qualitative results for 25 tests of pseudoephedrine with the Baeyer test showed positive results (Figure 6). These results were consistent with expectations. With these expectations being met the hypothesis was supported with a P-Value of 1 (df=1).

NIK Primary Test A Sugar	Negative Test For Amphetamines	Positive Test For Amphetamines
Observed	0	25
Expected	0	25
(Observed - Expected)^2 / Expected	~	0
X^2 Value	0	
Degrees of Freedom	1	
P-Value	1	

Figure 1: NIK test A results for sugar. The results were consistent with the expected results, giving a p-value of 1. The null hypothesis is supported.

NIK Secondary Test U Sugar	Negative Test For Methamphetamine	Positive Test For Methamphetamine
Observed	25	0
Expected	0	25
(Observed - Expected)^2 / Expected	~	25
X^2 Value	25	
Degrees of Freedom	1	
P-Value	0.00001	

Figure 2: NIK test U results for sugar. The results were not consistent with the expected results, giving a p-value of 0.00001. The null hypothesis must be rejected.

NIK Primary Test A Pseudoephedrine	Negative Test For Amphetamines	Positive Test For Amphetamines
Observed	0	25
Expected	0	25
(Observed - Expected)^2 / Expected	~	0
X^2 Value	0	
Degrees of Freedom	1	
P-Value	1	

Figure 3: NIK test A results for pseudoephedrine. The results were consistent with the expected results, giving a p-value of 1. The accuracy of the test was supported.

NIK Secondary Test U Pseudoephedrine	Negative Test For Methamphetamine	Positive Test For Methamphetamine
Observed	25	0
Expected	25	0
$(\text{Observed - Expected})^2$ / Expected	0	~
X^2 Value	0	
Degrees of Freedom	1	
P-Value	1	

Figure 4: NIK test U results for pseudoephedrine. The results were consistent with the expected results, giving a p-value of 1. The accuracy of the test was supported.

Baeyer Test Sugar	Negative Test for Double Bonds	Positive Test for Double Bonds
Observed	25	0
Expected	25	0
$(\text{Observed - Expected})^2$ / Expected	0	~
X^2 Value	0	
Degrees of Freedom	1	
P-Value	1	

Figure 5: Baeyer test results for sugar. The results were consistent with the expected results, giving a p-value of 1. The null hypothesis is supported.

Baeyer Test Pseudoephedrine	Negative Test for Double Bonds	Positive Test for Double Bonds
Observed	0	25
Expected	0	25
$(\text{Observed - Expected})^2$ / Expected	~	0
X^2 Value	0	
Degrees of Freedom	1	
P-Value	1	

Figure 6: Baeyer test results for pseudoephedrine. The results were consistent with the expected results, giving a p-value of 1. The accuracy of the test was supported.

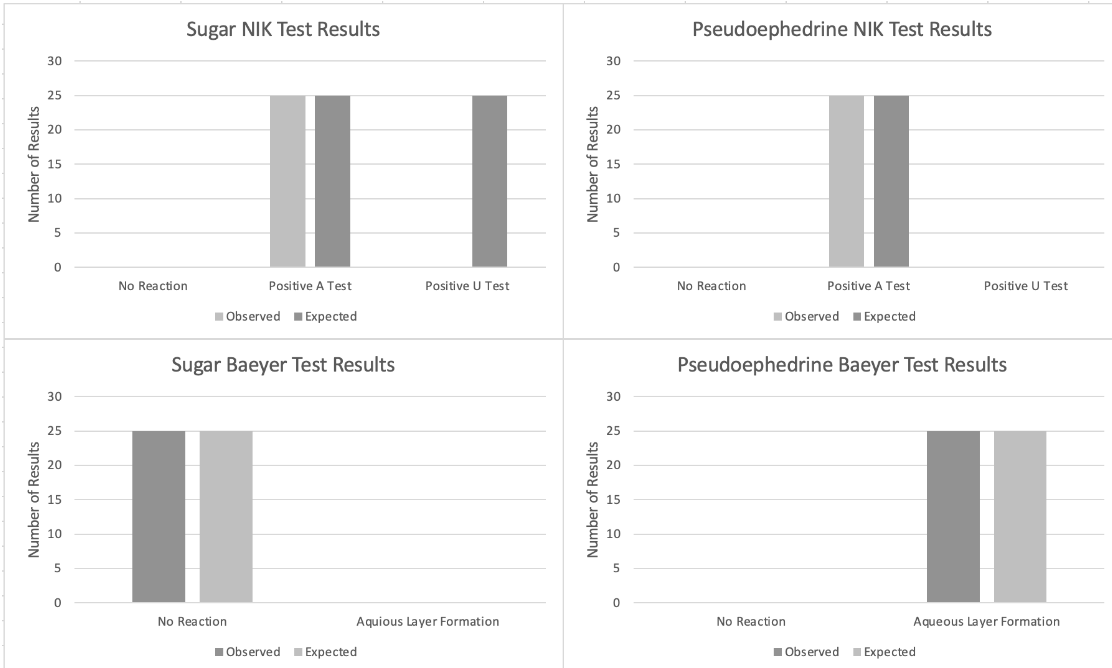

Figure 7: Column graph representations of the data collected from each of the chemical tests.

Discussion

With these results, it becomes clear that sugar tests positive for amphetamines using NIK Test A. This is of great concern given the inconsistency of sugar being similar to methamphetamine in a legal context given the purpose of NIK Test A. Indeed, sugar testing positive for presumptive tests used for amphetamines could lead to false arrest and placement into police custody until proper tests of the substance can be yielded.

Consistency in these results was compared to the known amphetamine pseudoephedrine which tested positive for amphetamines but negative for methamphetamine. Using these tests to show consistency in the prescribed results to be expected from NIK test A and U means that our results of sugar are consistent with expectations when using these tests. This eliminates any idea of these tests malfunctioning as the expected results were met.

Of interest with these results is that sugar tested positive for amphetamine presence but negative for methamphetamine presence. It was to be expected that if sugar gives off a false positive it should have consistent results with both of these tests. Of note here is that a negative result may be yielded with NIK Test U, but not NIK Test A, and with that inconsistency, in tests, there is room for error in false arrests if presumptive testing is to be used.

The inclusion of the Baeyer test indicates that this test could be used to differentiate the two compounds when the NIK tests could not. The Baeyer test showed negative results for sugar but positive results for pseudoephedrine, and when used in conjunction with the results in the NIK tests, determinations of inconsistencies can be made and analyzed. The Baeyer test is a cheap and effective way to differentiate the double bond nature that is present in amphetamines and not present within sugar. With proper usage of this test, false arrests can be avoided on the spot when checking between the NIK tests and the Baeyer test.

False positives are not new in presumptive testing, as many examples exist within the literature, with amphetamine false positives being very common (Brahm et al., 2011; Sanders and Gabrielson, 2016). False positives obviously raise many questions regarding the validity of presumptive testing, and how useful these tests truly are to law enforcement. As is frequently reported, the most valid method for confirmatory tests appears to be Mass Spectroscopy by most drug enforcement agencies and labs (Harper et al., 2017). Being aware of the shortcomings of presumptive drug testing is an important concern, and as such has been raised by many agencies (NES, inc Staff, 2017).

New developments are occurring to counteract these trends with drug testing results bringing back false-positive results (Choodum et al., 2014; Kim et al., 2015; McGeehan and Dennany, 2016). These current methods involve fluorescence assays for confirmatory results for drugs, but still, questions remain as to how practical and cost-effective new methods for drug testing can be.

Another proposed and easy test that can be used in conjunction with the already available NIK ampule tests could be a new Bayer ampule test. False positives can be checked with this simple, cheap, and effective test to check already positive results that have been shown in this report to give false positives. Applying this solution to an ampule test similar to the NIK ampule tests should be cost-effective and cheap to produce, giving one solution to the issues raised here about false positives.

Although our results seem to suggest continued efforts to combat false positives, our sample size is low, and more data needs to be collected across the united states about actual false positives recovered from drug enforcement, and subsequently analyzed using confirmatory testing to show no presence of amphetamines. Through various news outlets, this issue is abundantly clear, but there remain gaps in data about the prevalence of these occurrences (Sanders and Gabrielson, 2016). Furthermore, only sugar as a substrate that may be mistaken as amphetamine by NIK ampule tests was analyzed here. Many other substrates may exist that could lead to false positives, and with national data on these false-positive compounds a better picture could be gathered to see what kinds of compounds are more common to yield false positives.

Lastly, NIK ampule tests are also used for many other drugs giving law enforcement a means for presumptive testing. Protocols and certification for law enforcement on how to correctly use these tests should be followed or implemented, but even with proper training certain issues may still exist. Other issues that exist with presumptive testing have been shown to be present in opioids and cocaine, especially when used in urine analysis (Grates et al., 2008; Anderson, 2005; Saitman et al., 2014; Boyd and Sadrzadeh, 2019).

It is recommended here that the issues within presumptive testing are of obvious concern and should be recognized by law enforcement agencies regarding drug enforcement. The great societal concerns with drug abuse have given rise to new presumptive tests for the many drugs deemed illegal by society. With that comes the concern for the chance of false positives and many issues have already been identified and recorded with wrongful apprehension awning confirmatory results for various citizens through traffic stops. New methods for counteracting common false-positive substrates should be further analyzed, and with more data and recorded instances of false positives, a certain method to check for false positives in the field can minimize the many mistakes made in the cases of false arrest because of false-positive presumptive testing.

Conclusion

The data supports the hypothesis that sugar consistently tests positive for amphetamines when a Marquis test is administered. However, the data does not support that sugar consistently tests positive for methamphetamine. While it does not test positive for methamphetamine, a police officer would still have the right to take someone into custody if the test came up positive for amphetamines. Ideally, sugar would not test positive for either one of these outcomes, however, the data was consistent. Fortunately, the Baeyer test did prove to consistently determine that the sugar was not an amphetamine.

During none of the 25 administered Baeyer tests on sugar did an aqueous layer form, indicating that the substance being tested did not have any double bonds. Meanwhile, the pseudoephedrine molecule formed an aqueous layer every single time it was tested. Therefore, the test would be an effective secondary preliminary test to ensure that the sample being tested was an amphetamine.

Further research into this should be conducted to ensure that these results are accurate and consistent. The sample size must be increased to definitively support or reject the results of this initial study. This replication study would also be ideally conducted with multiple forms of sugar, to ensure that the common sugar is not the only kind that causes false positives. In addition, the ideal control chemical to use would be methamphetamine. If methamphetamine were to be the chemical control used, the U test would be confirmed as effective in the same way that pseudoephedrine confirmed the Marquis test to be effective.

As it stands though, the results were as conclusive as they could have been. At no point did we have any observable false positives or negatives, given that all of the results were consistent. As a result, the chi-squared analysis led to almost consistent p-values across all of the tests. With the exception of the NIK U test on sugar, all of the results were consistent with our chemical expectations.

The Baeyer test could be easily transformed into a standard field test much in likeness to a NIK test. All that would be required would be a pre-prepared glass ampule of acetone, and a dropper for potassium permanganate. The results would be easy to determine, the sample size needed would be low, and the results would come back fast. The test would also be inexpensive to produce, and easy to learn how to use. Overall, it would be an effective addition to police field testing practices for methamphetamine.

Supplemental Pages:

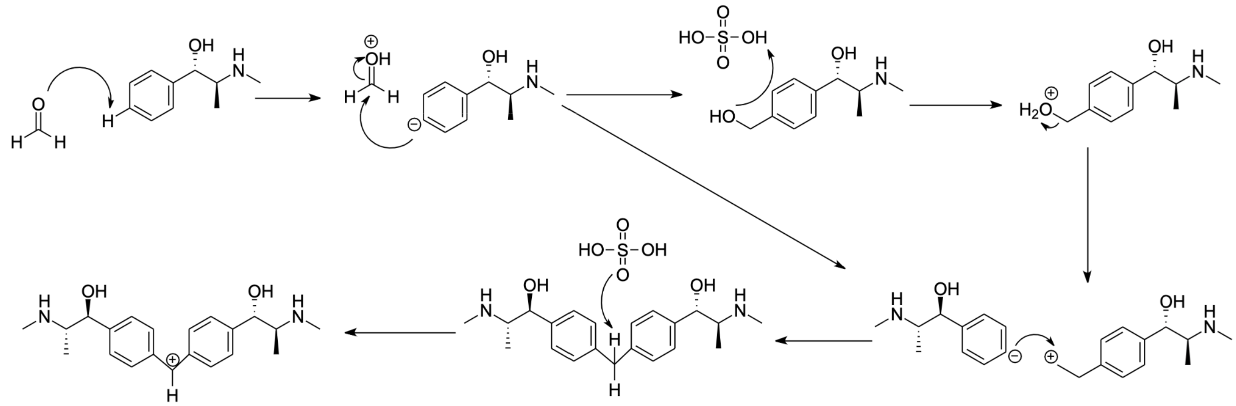

Figure 8: The theoretical arrow pushing mechanism for pseudoephedrine as it would react with the Marquis reagent.

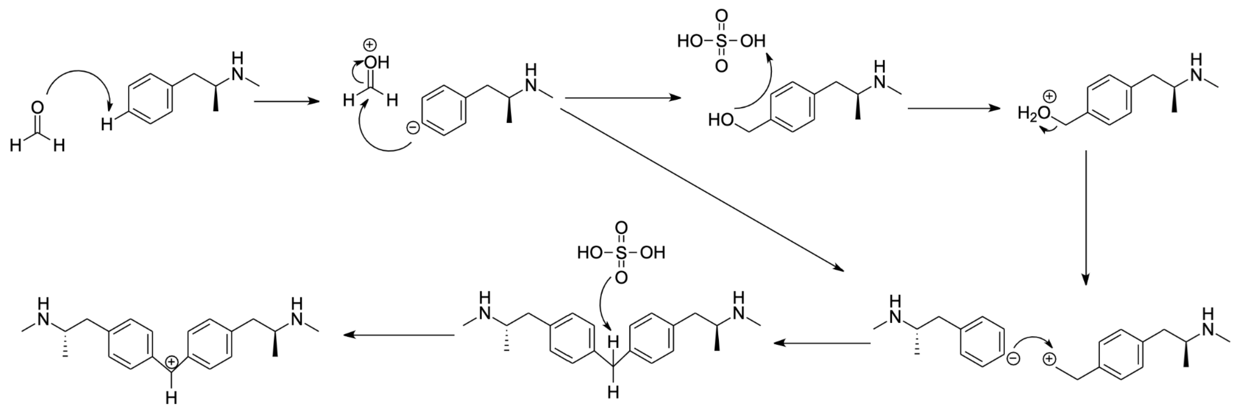

Figure 9: The theoretical arrow pushing mechanism for methamphetamine as it would react with the Marquis reagent.

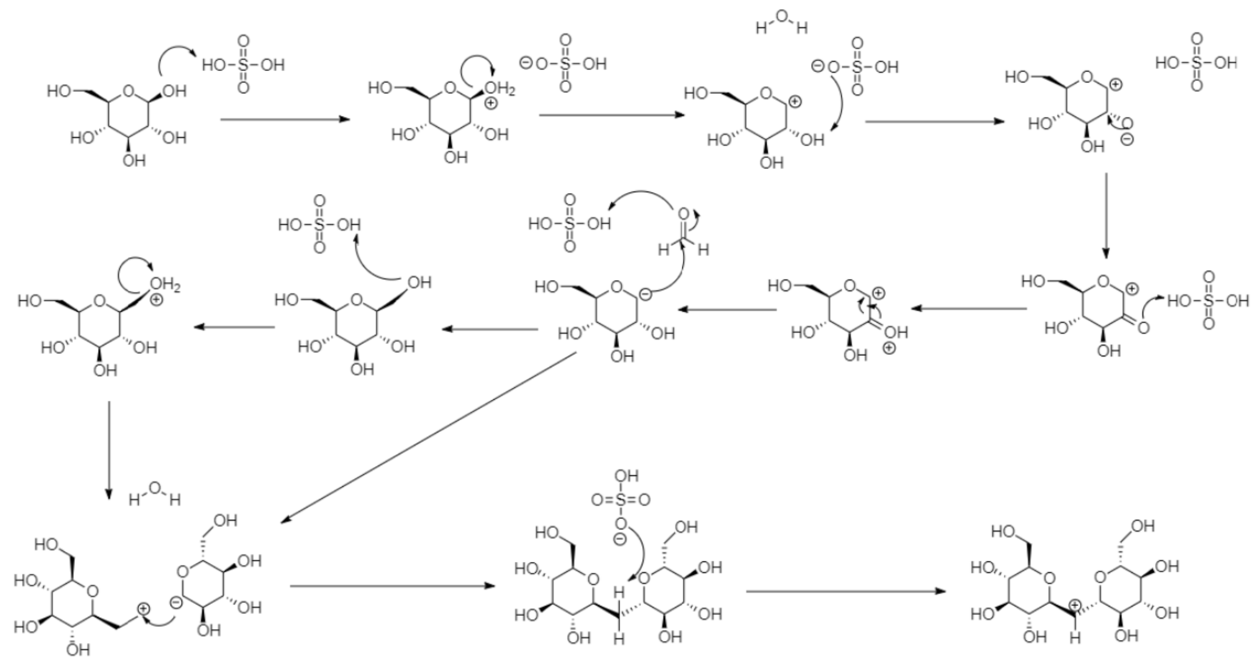

Figure 10: The theoretical arrow pushing mechanism for glucose as it would react with the Marquis test reagent.

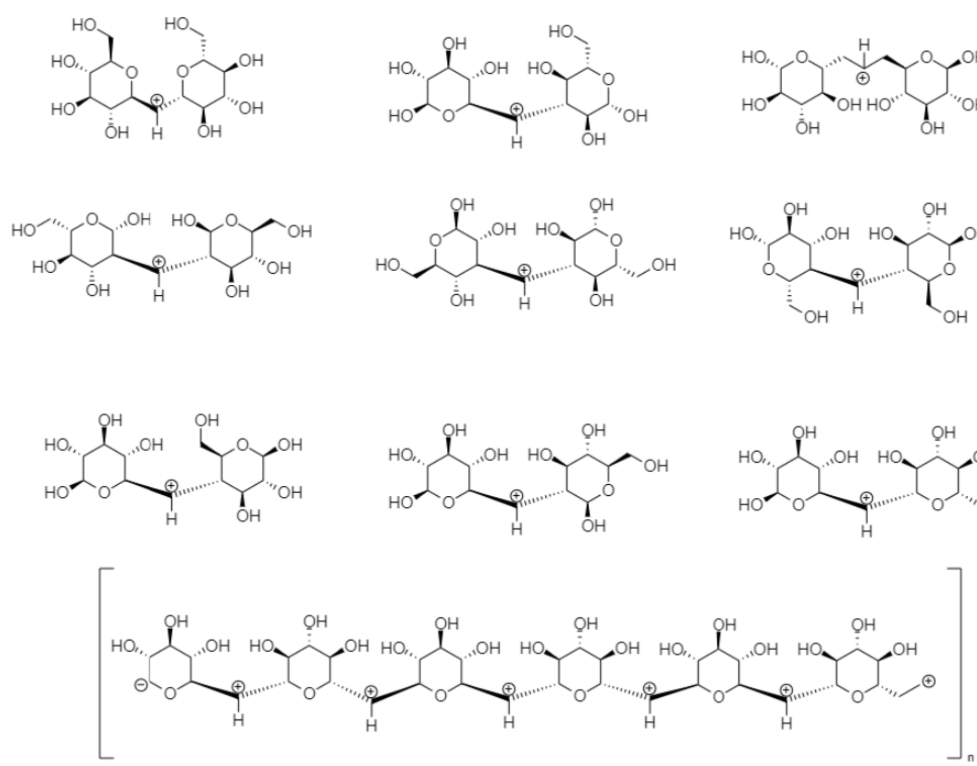

Figure 11: A small sample of the potential sugar complexes that can form as glucose molecules react with the Marquis reagent.

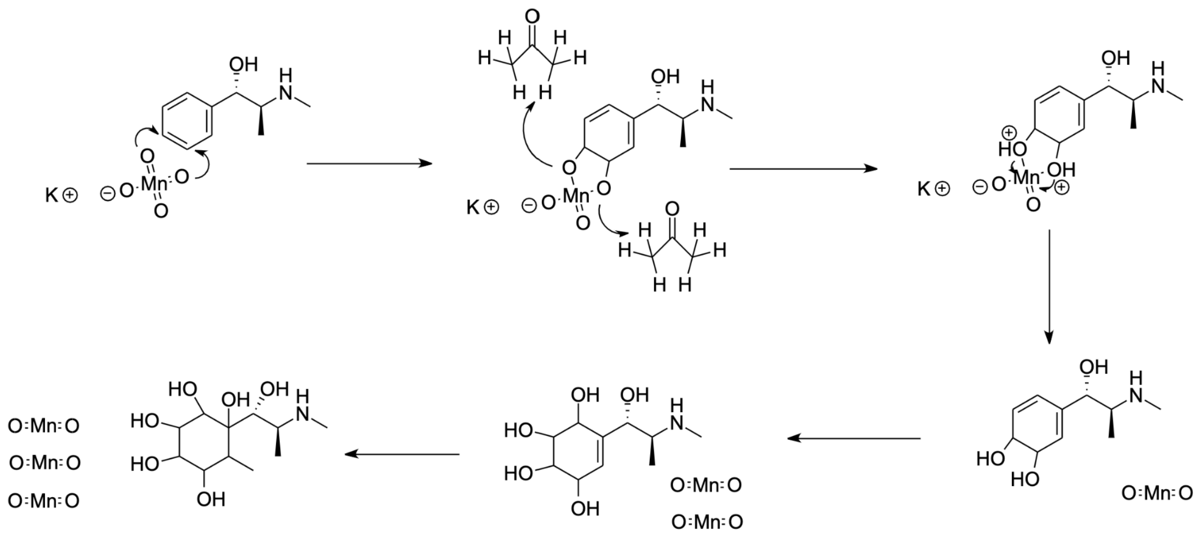

Figure 12: The theoretical arrow pushing mechanism for pseudoephedrine as it would react with the Baeyer test.

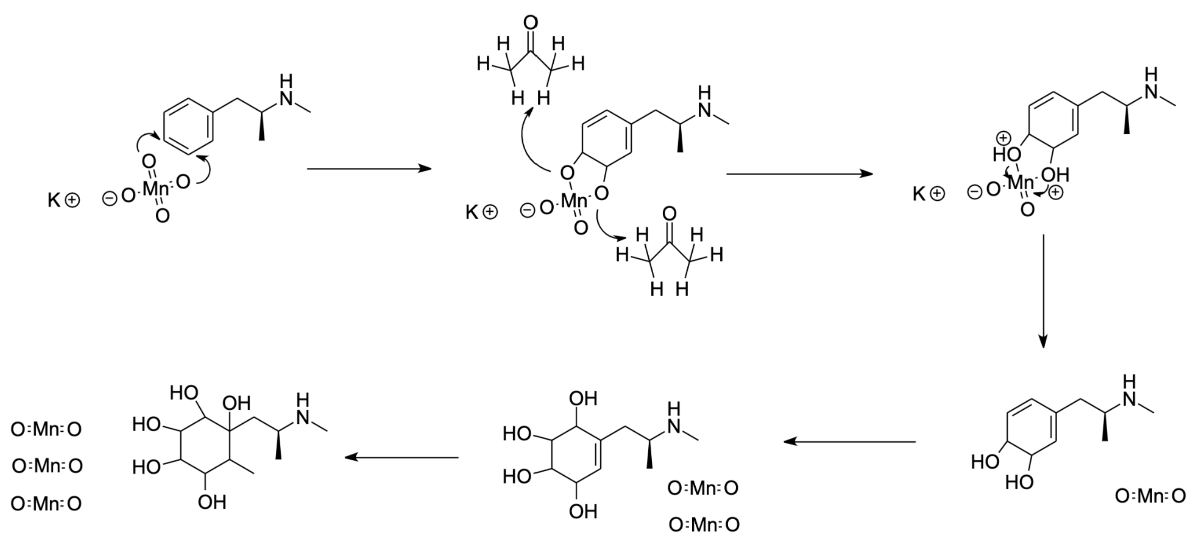

Figure 13: The theoretical arrow pushing mechanism for methamphetamine as it would react with the Baeyer test.

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