Discovery of an Interesting Temperature Effect on the Sensitivity of the Cobalt Thiocyanate Test for Cocaine

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ABSTRACT: During investigation of the mechanism and specificity of the Scott's (cobalt(II) thiocyanate) test for cocaine, it was discovered that the ambient temperature affected the equilibrium between the pink (negative) and the blue (positive) test results. At 4^{O}C (~39^{O}F) the sensitivity of the test was doubled versus room temperature (22^{O}C (~72^{O}F)), while temperatures in excess of 40^{O}C (~104^{O}F) decreased the sensitivity of the test more than twofold versus room temperature. These findings can impact the storage, use, and interpretation of commercially available cocaine test kits in typical field settings that are experiencing very cold or (especially) very hot ambient temperatures. A number of recommendations are offered to minimize the effects of hot temperatures on the test kits.

KEYWORDS: Cocaine, Scott's Test, Cobalt Thiocyanate, Presumptive Test, Color Test, Sensitivity, Temperature, Forensic Chemistry.

Introduction

The use of presumptive color tests in forensic and analytical laboratories to screen drug submissions is common [1]. Because of their ease of use and interpretation, a number of presumptive color tests for commonly submitted drugs have been incorporated into portable test kits for use by law enforcement personnel in field settings.[2] These test kits are mass-produced by a number of commercial manufacturers, and typically consist of one or more small ampoules of reagents in self-contained pouches that are reasonably priced, convenient, and safe to use. The results are easily interpreted, and the used kits are easily disposed of in accordance with hazardous waste statutes. Typically, a suspected controlled substance is placed into a tube or a pouch prior to breaking a glass ampoule containing a solution of a test reagent, agitating the mixture, and observing the results (usually an obvious color change). In more complex kits, a series of ampoules is broken in sequence, and the intermediate results at each

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[1] Current Address: Custom Sensors and Technology, 531 Axminister Drive, Fenton, MO 63026.

[2] Most such kits are based on chemical tests; more recently, a number of kits based on immunoassay testing have been produced (the latter are not further addressed in this study).

step dictate whether to continue to completion. Virtually all such kits come with instructions and color charts that show the expected color(s) for positive test results, and law enforcement personnel are well trained in their use.

A positive test result is considered to be a presumptive identification for the controlled substance that was being tested for, and it would be submitted to the laboratory as such. In addition to being a preliminary identification for laboratory analysis, a positive field test is also valuable as probable cause for an arrest, a further search incident to an arrest, and/or a search warrant. Furthermore, positive field tests ease the pressure on the judicial system, as defendants very commonly plead out during preliminary hearings when faced with presumptive identifications.

However, because of the wide variety of illicit drugs with similar appearances, virtually any suspect material would almost certainly be submitted (as an unknown/suspected controlled substance) even if the field test gave a negative or inconclusive result - especially if the appearance or packaging of the material, or the circumstances of the seizure, suggested that it was a controlled substance. However, a negative or inconclusive field test would likely result in a rush analysis request to the laboratory (especially likely if suspects were being detained pending the results), which is disruptive to laboratory operations. In addition, negative/inconclusive tests can encourage guilty defendants ("guilty" in this context meaning they are fully aware of the actual identity of the suspect material) to vigorously contest judicial proceedings until the results of analysis are returned from the laboratory. Thus, reliable and accurate tests are critical.

Cobalt Thiocyanate Test for Cocaine

The cobalt thiocyanate test for cocaine was first introduced by Young in 1931 [2]. The original test employed a two percent aqueous solution of cobalt(II) thiocyanate and tin(II) chloride in an aqueous hydrochloric acid solution. A positive test displays a blue color, while a negative test remains pink (i.e., the color of the test reagent). Even from its introduction, Young and others recognized that this test, while useful for rapid presumptive testing of cocaine, was not specific [3]; i.e., various other compounds gave "positive" results when subjected to this test. A number of variations of the test have subsequently been reported, virtually all focused on improving its specificity and/or sensitivity. Accounts of the evolution of the various versions of this test, with their relative advantages and disadvantages, are well-documented in the literature [4].

The most commonly employed current incarnation of this test, known as the Scott's test [4e], employs a three-stage sequence: 1) A 1:1 water/glycerine solution of cobalt thiocyanate is added to the suspect substance (resulting in a blue precipitate and a blue solution); 2) Concentrated hydrochloric acid is added (the blue precipitate dissolves and the liquid turns pink); and 3) Chloroform is added (the upper (aqueous) layer remains pink, while the lower (chloroform) layer turns blue). A positive result at each stage is required in order to qualify as a positive test for cocaine. This sequence is not only more specific for cocaine, but can also detect both cocaine base and cocaine hydrochloride. However, despite its significantly improved specificity versus the original (Young) test, the Scott's test is still subject to false positive and false negative results, which have inspired continuing modifications and alternative tests. Nonetheless, its convenience and utility as a presumptive test in the hands of trained personnel have made it a mainstay in the arsenal of qualitative forensic reagents, and it is the basis for most (if not all) chemistry-based field test kits for cocaine.

Not surprisingly, the Scott's test is one of the most frequently performed field tests. As noted above, cocaine test kits that are based on the Scott's test are commercially produced by several different manufacturers; however, all are similar in their design and use. Typically, a qualitatively prescribed amount of the suspected cocaine is placed inside a thick transparent plastic pouch containing three secured ampoules, and the pouch is sealed. The first ampoule, containing the cobalt thiocyanate solution, is broken, the mixture is agitated, and the color and precipitate (if any) are noted. The second ampoule, containing the hydrochloric acid solution, is broken, the mixture is further agitated, and the color is again noted. Finally, the third ampoule, containing chloroform, is broken, again with agitation and observation of the colors in the two layers [5]. A positive result at each of the three stages is considered to be a presumptive identification of cocaine. Anything less than a positive result at any stage is considered to be inconclusive or negative.

However, a positive test does not confirm cocaine, and a negative test does not mean that cocaine is not present. As noted above, a number of other compounds give a “positive” test result (thus giving what is typically referred to as a “false positive”). Still other compounds can interfere with a positive result (giving a “false negative”). In addition, the test is sensitive to the quantity of test material used - both insufficient and excessive quantities of cocaine are documented to produce false negatives [4e]. The potential for chemical interference(s), and the sensitivity of the test to the quantity of cocaine present, are especially important considering the fact that illicit cocaine is almost always cut with other substances at the retail and wholesale levels, and (increasingly) even at the production level (currently (2008), it is common for cocaine kilogram bricks produced in South America to be adulterated with small to moderate percentages of diltiazem, hydroxyzine, or levamisole [6]). Adulterants and diluents can not only interfere with the test, but also decrease the actual amount of cocaine placed in the test kit.

Mechanistic Studies of the Cobalt Thiocyanate Test

Efforts to better understand the cobalt thiocyanate test and its limitations have been aimed at two primary areas of study: 1) Elucidation of the mechanism of the test [7]; and 2) Empirical documentation of compounds giving false positive or interfering tests. While an abundance of useful information exists in the forensic literature for each of these two areas of study, a rigorous explanation of the mechanism of the test remains elusive.

As noted above, a positive test displays a blue color in Step 1, while a negative test retains the pink color of the test reagent. Although various hypotheses have been published, the exact structures of the blue and pink complexes are unknown. It is known that octahedral cobalt(II) complexes, such as $[Co(H_2O)_6]^{2+}$, are typically pink, whereas tetrahedral cobalt(II) complexes like $[CoCl_4]^{2-}$ are typically an intense blue [8]. Ligand field theory explains these phenomena quite well. Critically, these changes in geometry are reversible and are often accompanied by enthalpy changes. For example, the equilibrium of octahedral and tetrahedral cobalt complex ions in the presence of chloride anion may be expressed as follows [9]:

(Eq. 1) $[CoCl(H_2O)_5]^+(aq) + Cl^-(aq) \rightarrow CoCl_2(H_2O)_2(aq) + 3\ H_2O(l)$ $\Delta H \sim 48$ kJ/mole

Since the forward reaction is endothermic (i.e., it requires heat as a reactant), raising the temperature favors the formation of the blue tetrahedral complex, while lowering the temperature favors the formation of the pink octahedral complex. This phenomenon is an excellent demonstration of Le Chatelier’s principle [10].

A reasonable inference would be that a similar change in cobalt geometry occurs in the Scott’s test. The basic equation for this analogous equilibrium (i.e., substituting thiocyanate for chloride) can be expressed as follows:

(Eq. 2) $[Co(SCN)(H_2O)_5]^+(aq) + 3\ SCN^-(aq) \rightarrow [Co(SCN)_4]^{2-}(aq) + 5\ H_2O(l)$ $\Delta H \sim ?$ kJ/mole

However, in this case the exact structure of the pink octahedral and the blue tetrahedral species are unknown, and it is likely that gradual replacement of aqua with thiocyanato ligands gives a range of pink and blue colored intermediate species.

In the absence of cocaine, the equilibrium would be expected to lie to the left in an aqueous solution (where the excess water competes for the coordination sites on the cobalt). However, if cocaine is present, the substitution of two bulky, relatively hydrophobic protonated cocaine cations in the coordination sphere lends stability to the complex, rendering it more soluble in organic solvents (such as chloroform), favoring the right side of the equilibrium. That is, the cocaine serves to partly or fully exclude water from the coordination sphere, causing the equilibrium to shift to the right. Thus, the blue coordination compound responsible for a positive Scott’s test might be $(R_3NH)_2Co(SCN)_4$, where R_3NH^+ represents the protonated cocaine molecule [7], as follows:

(Eq. 3) $[Co(SCN)(H_2O)_5]^+(aq) + 3\ SCN^-(aq) + 2\ R_3NH^+ \rightarrow (R_3NH)_2Co(SCN)_4 + 5\ H_2O(l)$

Surprisingly, no studies examining the effect(s) of temperature on this equilibrium have been reported in the forensic literature. Le Chatelier's principle predicts that applying thermal stresses to the equilibrium system will affect its position, favoring either the reactants or the products, and thereby altering the sensitivity of the test. Thus far, published studies of the sensitivity and specificity of the Scott's test have focused on the presence of interfering analytes giving false positive or false negative results, on substituting different acids and organic solvents in the test, or on the quantities of cocaine used in the test kits [4], but none have considered temperature.

Furthermore, the various manufacturers of the test kits do not specifically address this issue in their product literature. One of the test kit manufacturers does state (in a newsletter) that "a cold test will simply show the color reactions slower than a room temperature test" [11]. However, the same newsletter also states that "[f]ield tests can be stored without concern in any container (desks, briefcases, cabinets, glove compartments, or vehicle trunks)." In view of the thermodynamics of the cobalt complexes, these assertions appear dubious.

Test kits are typically stored in vehicles prior to use, where (depending on locale) temperatures can fall below freezing during winter months and can exceed $140^{O}F$ ($60^{O}C$) during summer months. Two critical questions are: 1) Do the temperatures commonly achieved inside the cabin or trunk of a vehicle during winter or summer months significantly affect the sensitivity of the Scott's test?; and 2) Can temperature-controlled storage be used to enhance the sensitivity of the Scott's test?

Experimental

[*Editor's Notes: Because publication in <u>Microgram</u> <u>Journal</u> could be interpreted as an endorsement or a counter-endorsement by the U.S. Drug Enforcement Administration, the names of the test-kit manufacturers and the names of their test kits have been redacted from this article. The results apply equally to virtually any Scott's test-based test kit.*]

Cobalt(II) thiocyanate was purchased from Sigma-Aldrich. Concentrated hydrochloric acid and chloroform were purchased from Fisher Scientific. Reference standard cocaine was purchased from Sigma-Aldrich and was stored and used in the drug chemistry section of the Missouri State Highway Patrol Troop E Satellite Laboratory. Cocaine test kits used for this study were purchased from a well known manufacturer of narcotics field test kits, and were stated to be applicable for testing both cocaine salts and cocaine base. The provided instructions for use were followed, including a printed qualitative sample size indicator. Reduced temperature studies were conducted using a standard portable cooler with ice as the cooling agent. Elevated temperature studies were conducted using a standard laboratory oven.

Stock Solution Studies

A preliminary test was done to confirm that the equilibrium reaction operating in the Scott's test for cocaine could in fact be manipulated by temperature. For economy, a stock solution of cobalt thiocyanate was prepared to closely match the concentration of the solution contained in the test kit ampoules. A series of calibration solutions was prepared such that the concentration of the test kit solution was within the concentration range of the calibration set, as judged by visual inspection. The actual absorbances of the solutions - measuring 0.05, 0.10, and 0.15 M in concentration - were formally measured at 517 nanometers using a Beckman DB-G UV-Vis spectrophotometer.

Using the resulting calibration curve and measuring the absorbance of the test kit solution from the ampoule, it was determined that the test kit solution was approximately 0.11 M cobalt(II) thiocyanate. A stock solution at this concentration was prepared and used in preliminary tests with various quantities of cocaine and at room, reduced, and elevated temperatures.

Reduced-Temperature Studies (with Test Kits)

A number of test kits were cooled to approximately 4^{O}C in a small insulated cooler. While these pouches were being cooled, a benchmark test was performed using a kit at room temperature, following the manufacturer's instructions printed on the product box. The quantity of cocaine prescribed by the circle printed on the box was measured visually and then weighed using a laboratory balance (mass = 0.5 mg). This sample was then placed into the control test kit, and the series of ampoules was broken in sequence with agitation and observation in accord with the instructions. This test result was noted and was the basis of comparison for all subsequent tests.

The quantity of cocaine (0.5 mg) and the procedures used for the benchmark test were then duplicated for a test pouch cooled to 4^{O}C (39^{O}F). Following this test, a second reduced-temperature test was performed using a quantity of cocaine approximately one-half the size of the recommended amount used in the initial test (0.2 mg). The quantity of cocaine was cut in half again, to approximately one-fourth the size recommended by the manufacturer (0.1 mg), and the test procedure was repeated. Duplicate tests were run to confirm each result. Trials were also performed to compare the effect of pre-cooling the test kits prior to introduction of the cocaine samples versus attempting to cause a positive or negative result to be reversed by cooling the test kits after a result was obtained at room temperature for a given sample.

Elevated-Temperature Studies (with Test Kits)

A number of test kits were warmed to either 45^{O}C (113^{O}F) or 60^{O}C (140^{O}F) in a standard laboratory oven. Another benchmark test was performed at room temperature and was used as a reference in the elevated-temperature study. The quantity of cocaine (0.5 mg) and the procedures for the benchmark test were then duplicated for test pouches warmed to 45^{O}C and 60^{O}C. Following each of these tests, a second set of elevated-temperature tests was performed using a quantity of cocaine approximately double the size of the recommended amount used in the initial test (1 mg).

Results and Discussion

Stock Solution Studies

Initial investigations revealed that 1 milligram of cocaine, combined in a test tube with one drop of concentrated hydrochloric acid and two drops of 0.11 M cobalt thiocyanate stock solution, resulted in the formation of blue flakes and a blue solution - a positive test. Based on literature data for the aqua complexes of cobalt(II) chloride, it was expected that decreasing the temperature of this positive test solution would shift the equilibrium toward the pink octahedral complex responsible for a negative test, while increasing the temperature would have the opposite effect. In actuality, however, cooling the blue solution to 4^{O}C (39^{O}F) had no significant impact on the color, while raising the temperature resulted in a change from blue to pink. At only 30^{O}C (86^{O}F), the solution began changing from blue to pink with blue specks. Continued heating to 60^{O}C (140^{O}F) resulted in a pink solution with no blue specks.

Next, the mass of cocaine was decreased to less than 0.5 milligram. This quantity was insufficient to produce a blue color in the test tube at room temperature. Raising the temperature of this solution gradually to 60^{O}C (140^{O}F) did not produce any significant changes in its appearance. However, cooling the solution to 4^{O}C (39^{O}F) resulted in a change in color from pink to blue - a positive test. Collectively, these results suggest that the reaction responsible for the change from pink to blue color in the Scott's test for cocaine is exothermic with a ΔH on the same order of magnitude (but of opposite sign) as that for the endothermic reaction involving cobalt(II) chloride (shown in Eq. 1). The results also indicate that the temperature of a field test kit for cocaine will significantly impact the sensitivity and accuracy of its response.

Reduced-Temperature Studies (with Test Kits)

Results are summarized in Table 1. Interestingly, in one trial, the benchmark test, using 0.5 milligram of cocaine at room temperature, yielded a negative result (in that Step 3 failed to produce the requisite blue lower chloroform layer). This indicates that there is little tolerance for error in the manufacturer's instructions for this particular test kit. In actual practice, this result would likely prompt re-testing with a larger quantity. However, cooling the negative test pouch to 4^{O}C (39^{O}F) resulted in a blue coloration in the chloroform layer, indicating a positive test for cocaine. Furthermore, cooling a new test kit pouch to 4^{O}C prior to re-testing at the 0.5 milligram level resulted in a noticeably stronger positive test result at all three stages, further confirming enhanced sensitivity at lower temperatures.

To further study this finding, the quantity of cocaine was cut to approximately 0.2 mg. Testing this amount with a pouch that had been pre-cooled to 4^{O}C again resulted in a positive result at all three stages. However, when the sample size was reduced to 0.1 mg, Steps 1 and 2 gave positive tests, but the chloroform layer did not develop a blue color in Step 3.

These results stand in contrast to the statement made in the manufacturer newsletter [11], and they confirm that storing the test kit at low temperatures - either incidentally due to weather conditions or intentionally in a portable cooler - increases its sensitivity by more than a factor of two. The results also show that cooling a test pouch that was positive at Steps 1 and 2 but negative at Step 3 is not as effective as pre-cooling it to 4^{O}C prior to testing.

Elevated-Temperature Studies (with Test Kits)

Results are summarized in Table 2. Since the benchmark test was already at the limit for a repeatable positive test, it was expected that a relatively small temperature increase would result in a negative test - and in fact pre-warming the test kits to 45^{O}C (113^{O}F) gave negative results at all three stages. Not surprisingly, increasing the temperature to 60^{O}C (140^{O}F) also gave negative results. These temperatures are routinely attained inside a parked vehicle during warm weather, especially if the vehicle is exposed to the open sun, and even if the windows are slightly opened for ventilation.

To attempt to compensate for the reduced sensitivity observed at higher temperatures, the sample size was increased from 0.5 mg to 1 mg. In the 45^{O}C (113^{O}F) trials, Step 1 yielded a pink solution with no blue flakes. Continuing the test procedure through Step 3 (despite the test instructions dictating that the test be terminated after a negative result for Step 1), a faint blue chloroform layer was observed. Anyone following the test instructions, however, would never have reached this stage. Again not surprisingly, increasing the temperature to 60^{O}C (140^{O}F) still gave negative results with 1 mg. This confirms that elevated temperatures decrease the sensitivity of the Scott's field test at least twofold.

Transient False Positives at Elevated Temperatures

Elevated temperatures, in addition to producing false negatives, had a further complicating factor. At both 45^{O}C and at 60^{O}C, a transient blue solution was observed at Step 2, which persisted for a few seconds after mixing and agitating. This transient coloration is also often observed at room temperature, just after breaking the second ampoule, even in the absence of cocaine. Presumably it is due to a temporary and localized high concentration of chloride ions prior to complete mixing of the pouch contents, but at room temperature it dissipates very rapidly with agitation. At 45 and 60^{O}C, however, due to the thermodynamic equilibrium of the cobalt(II) chloride reaction (Eq. 1), this color is more intense and persists for a much longer time. In fact, at 60^{O}C, even up to one minute after the initial intense blue color dissipates, the color of the solution can best be described as pink to lavender, rather than pink. This is presumably due to a sufficient concentration of the blue tetrahedral chloro complex, or of one or more of the intermediate (i.e., partially chlorinated) complexes, imparting a blue tint to the otherwise pink solution. This result is obviously directly attributable to the temperature of the pouch when used, and again could potentially lead to false positive or inconclusive tests.

Safety Issues at Elevated Temperatures

In addition to the loss of sensitivity and accuracy at elevated temperatures, two safety considerations were noted: First, the plastic clip that was used to seal the pouches (intended to prevent leakage of the reagents during agitation) can be deformed by elevated temperatures (45 - 60^{O}C), potentially allowing the pouch to open accidentally or leak during agitation, spilling hazardous chemicals. A second concern is that chloroform boils at 61^{O}C. Therefore, breaking the third ampoule while the kit is over 60^{O}C will (at a minimum) result in pressurization of the pouch, and possibly cause a hazardous chemical aerosol spray to be emitted from an improperly sealed pouch. The authors are unaware of any literature reports of such problems, but the potential clearly exists.

Conclusions (and Recommendations)

The findings of this study suggest that better guidelines can and should be implemented for the storage and use of cocaine field test kits. The National Institute of Justice sets a generic standard for color test reagents and kits that recommends an ambient test temperature between 10^{O}C and 40^{O}C (50^{O}F and 104^{O}F) [12]. Based on the results of the presented study, the high temperature limit of 40^{O}C is clearly too high for cocaine field test kits, and can result in false negatives. Furthermore, although not as critical an issue, the low temperature limit of 10^{O}C is also too high, and needlessly sacrifices sensitivity.

Although the test kits can be stored and used at room temperature, storage at 4^{O}C (39^{O}F) is recommended to both enhance the sensitivity of the test and reduce the likelihood of false negatives due to low sample purity or user error. From a practical viewpoint, a single test kit can be cooled to close to this recommended temperature in approximately 10 minutes by clipping it to the front of a vehicle's dashboard vent while running the air conditioner at maximum cooling capacity (assuming the vehicle's A/C unit is properly operating). Maintaining a test kit clipped in this position at all times while on call would ensure the ready availability of a cooled test kit when needed, and is recommended in scenarios where law enforcement personnel have a reasonable but minor expectation of need. In cases where a larger volume of testing is anticipated, such as a planned search of a home, building, vehicle, boat, ship, or aircraft, etc., or where operational circumstances otherwise preclude using a vehicle's air conditioner, test kits may be maintained at 4^{O}C by storing them in a small, portable cooler with ice or cold-packs, or in a 12-volt powered portable thermoelectric cooler maintained at 39^{O}F (4^{O}C). In testing, it was determined that a pair of test kits can be cooled from 100^{O}F (38^{O}C) to 4^{O}C in 20 minutes in a typical thermoelectric cooler (i.e., intended for use in a vehicle), and can be maintained at that temperature indefinitely if the power supply is maintained. If necessary, large quantities of test kits could be stored in a designated chemical refrigerator at an appropriate facility, to ensure a constant supply of pre-cooled test kits that can be transferred as needed to portable thermoelectric cooler in vehicles.

Finally, besides yielding recommendations for the storage and transportation of cocaine field test kits, these findings also suggest the prudence of scrutinizing other field tests (i.e., for other illicit substances) to determine whether they have similar temperature sensitivity issues.

Future studies at the authors' laboratories include examination and quantitation of the effects of temperature on the response of known interferences in the Scott's test for cocaine; i.e., does use at 4^{O}C both increase the sensitivity of the test to cocaine but also to other substances that are already known to give false positives? Or (as may well be) does it further improve the specificity of the test for cocaine?

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Temperature	Mass	Step 1	Step 2	Step 3
22°C (72°F)	0.5 mg	pink solution blue flakes (+)	pink solution (+)	pink upper layer colorless lower (-)
22°C (72°F)	0 mg (-) control	pink solution no blue flakes (-)	pink solution (+)	pink upper layer colorless lower (-)
4°C (39°F)	0.5 mg	pink solution blue flakes (+)	pink solution (+)	pink upper layer blue lower (+)
	0.2 mg	pink solution blue flakes (+)	pink solution (+)	pink upper layer blue lower (+)
	0.1 mg	pink solution blue flakes (+)	pink solution (+)	pink upper layer colorless lower (-)
	0 mg (-) control	pink solution no blue flakes (-)	pink solution (+)	pink upper layer colorless lower (-)

Table 1. Results of Reduced-Temperature Study. Results are noted along with a (+) or (-) symbol to indicate a positive or negative inference. Test instructions dictate that the test ends after the first negative result. Procedure: Step 1: The first ampoule, containing the cobalt thiocyanate solution, is broken, the mixture is agitated, and the color is noted. A blue color in the solution or oily blue flakes indicates the possible presence of cocaine and permits advancement to the next step; Step 2: The second ampoule, containing the hydrochloric acid solution, is broken, the mixture is further agitated, and the color is again noted. A pink colored solution is observed but is not particularly diagnostic for cocaine; Step 3: The third ampoule, containing chloroform, is broken, again with agitation and observation of the resultant colored layers. A pink upper layer and a blue lower layer indicate the possible presence of cocaine.

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Temperature	Mass	Step 1	Step 2	Step 3
22°C (72°F)	0.5 mg	pink solution blue flakes (+)	pink solution (+)	pink upper layer faint blue lower (+)
45°C (113°F)	0 mg (-) control	pink solution no blue flakes (-)	pink solution (+)	pink upper layer colorless lower (-)
60°C (140°F)	0 mg (-) control	pink solution no blue flakes (-)	pink ~ lavender solution (?)	pink upper layer colorless lower (-)
45°C (113°F)	0.5 mg	pink solution no blue flakes (-)	pink solution (+)	pink upper layer colorless lower (-)
60°C (140°F)	0.5 mg	pink solution no blue flakes (-)	pink ~ lavender solution (?)	pink upper layer colorless lower (-)
45°C (113°F)	1 mg	pink solution no blue flakes (-)	pink solution (+)	pink upper layer faint blue lower (+)
60°C (140°F)	1 mg	pink solution no blue flakes (-)	pink ~ lavender solution (?)	pink upper layer colorless lower (-)

Table 2. Results of Elevated-Temperature Study. Results are noted along with a (+) or (-) symbol to indicate a positive or negative inference. A (?) symbol indicates an unpredicted observation that may be confusing or inconclusive to the field analyst. Test instructions dictate that the test ends after the first negative result. Procedure: See Table 1.

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