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TECHNICAL NOTE

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Modified Cobalt Thiocyanate Presumptive Color Test for Ketamine Hydrochloride

ABSTRACT: A new presumptive color test for ketamine hydrochloride is reported. The test is a modification of the cobalt thiocyanate test currently used for cocaine and involves basifying samples rather than acidifying them. The two-step procedure for liquids and three-step procedure for powdered samples are straightforward, definitive, and utilize reagents commonly used in forensic drug analysis. The test works on ketamine hydrochloride in both powder and liquid form and has a sensitivity of *c*. 1.25 mg. Performing the test with numerous other controlled substances and related chemicals demonstrates the test to be highly selective.

KEYWORDS: forensic science, ketamine, color test, screening test, presumptive test, cobalt thiocyanate

Ketamine, a Schedule III federally controlled substance, is routinely detected and confirmed by forensic laboratories. Analytical techniques used in analysis include infrared spectroscopy (1,2), gas chromatography/mass spectrometry (GC/MS) (1–5), ultraviolet spectroscopy (4,5), thin-layer chromatography (6), and presumptive color tests. Currently, only the Janovsky color test has been reported for presumptive ketamine detection (7). However, several benzodiazepines give the same color with the Janovsky reagent.

A new presumptive test for ketamine hydrochloride is reported below. The test is a modification of the standard cobalt thiocyanate (CoSCN) test for cocaine. The test is straightforward, definitive, and utilizes reagents commonly used in forensic drug analysis. The test works on ketamine hydrochloride in both powder and liquid form.

Standards and Reagents

Ketamine hydrochloride and all other controlled or noncontrolled substances were obtained from the analytical reference materials of the author's laboratory. Street samples of suspected ketamine were obtained from submitted cases. CoSCN was obtained from Aldrich (St. Louis, MO), and sodium hydroxide was obtained from Fisher (Fair Lawn, NJ). Deionized water was obtained from commercial deionization beds. All other chemicals used in the study were obtained from recognized commercial suppliers.

Reagent Preparation

CoSCN solution: Two grams of CoSCN was added to 100 mL of deionized water.

0.1 N NaOH solution: Two grams of NaOH was added to 500 mL of deionized water.

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Experimental Procedure

A two-step procedure for liquids and a three-step procedure for powered samples were utilized, each forming the same specific color formation for ketamine.

General Test Procedure for Liquid Samples

A single drop of the sample was added to either a spot well or a test tube, followed by a single drop of 0.1 N NaOH. A drop of the CoSCN solution was added with mixing. Samples containing ketamine formed a lavender to purple precipitate. A negative result was indicated by a dull blue–green precipitate. A blank was also performed by side-by-side comparisons of the blank and the sample-colored precipitates.

General Test Procedure for Powdered Samples

A small amount of sample (tip of a small spatula) was added to a spot well or a test tube, followed by a single drop of deionized water and a single drop of 0.1 N NaOH. A drop of the CoSCN solution was added with mixing. Samples containing ketamine formed a lavender to purple precipitate. A negative result was indicated by a dull blue–green precipitate. A blank was also performed by side-by-side comparisons of the blank and the sample-colored precipitates.

Results and Discussion

To the author's knowledge, modification of the CoSCN test by basifying samples before addition of the CoSCN has not been previously reported. The presented modification was discovered while attempting to enhance ketamine's color reaction to standard CoSCN presumptive tests. Our lab, like others with which we have communicated, found that powdered ketamine HCl turns blue to dark blue with neutral CoSCN. However, liquid ketamine samples do not produce the same color reaction. Acidifying the

samples, powder or liquid, only resulted in a pink solution with the typical CoSCN test.

Specificity

Ninety-four compounds covering a wide variety of controlled substance classes, adulterants, diluents, and excipients were screened using the modified CoSCN test. Table 1 lists the compounds tested and their individual results. Common solvents such as tap water, a saline solution, methanol, and ethanol were also screened. The modified CoSCN test revealed a surprising specificity to ketamine hydrochloride. Of all the compounds tested, only Hydrocodone, Levorphanol, Zolpidem (all tartrate salts), and Mepivacaine HCl gave a colored precipitate somewhat similar to ketamine hydrochloride's purple to lavender precipitate. Even then, the precipitate with these compounds was blue, easily distinguishable from that of ketamine hydrochloride. Most compounds gave a blue–green precipitate, which is the same result as a blank. Even phencyclidine, a structurally related compound, gave a precipitate (bright-blue) easily distinguished from that of ketamine hydrochloride.

Precipitates from ketamine hydrochloride, phencyclidine hydrochloride, and a blank deionized water solution were collected, dried, and analyzed by FTIR. Figure 1 compares the spectra of the ketamine hydrochloride, phencyclidine hydrochloride, and blank precipitates. The fact that these precipitates can be distinguished spectroscopically confirms the visual distinctions between the different precipitates.

Investigation of the Color Formation

Investigation into the source of the color development indicated an unknown cobalt complex involving ketamine, the thiocyanate ion, the hydroxide ion, and a chloride ion. Confirmation that the color was the result of a complex, rather than a permanent change of the ketamine molecule through a chemical reaction, was obtained when dissolution of the precipitate into methanol followed by GC/MS analysis resulted in a single component of unchanged ketamine.

TABLE 1—*Presumptive test results.*

1,4-Butanediol	—	Lidocaine	—
2,4,6-Trimethoxyamphetamine HCl	—	Mannitol	—
2,5-Dimethoxy-4-bromoamphetamine HBr	—	MDA HCl	—
2,5-Dimethoxy-4-ethylamphetamine	Blue ppt in pink sol'n	MDEA HCl	—
2,5-Dimethyoxy-4-methylamphetamine HCl	—	MDMA HCl	—
4-Methyoxyamphetamine HCl	—	Medazolam	—
5-Methoxy-α-methyltryptamine HCl	—	Meperidine HCl	—
5-Methoxy-*N*,*N*-dimethyltryptamine	—	Mepivacaine HCl	Blue ppt
Acetaminophen	—	Mescaline HCl	—
Alprazolam	—	Methadone HCl	Bright blue ppt
Amobarbital	—	Methamphetamine HCl	—
Amphetamine sulfate	—	Methapyrilene HCl	Bright blue ppt
Benzoyl ecogonine	—	Methaqualone HCl	—
Benzphetamine HCl	—	Methcathinone HCl	—
Butalbital	—	Methocarbamol	—
Cathinone HCl	—	Methylphenidate HCl	—
Chlordiazepoxide HCl	—	Modafinil	—
Citric acid, 3NA salt	Pink sol'n with faint blue ppt	*N*,*N*-Diisopropyl-5-methoxytryptamine HCl	Bright blue ppt
Clonazepam	—	*N*,*N*-Dimethylamphetamine HCl	—
Clorazepate, 2K salt	Light blue ppt	Naproxen	—
Cocaine base	—	N-Hydroxy-MDA HCl	—
Cocaine HCl	—	Nicotinamide	—
Codeine	—	Nitrazepam	—
Dextromethorphan HBr	—	Nordiazepam	—
Dextropropoxyphene HCl	Bright blue ppt	Opium	—
Dextrose	—	Oxazepam	—
Diazepam	—	Oxycodone HCl	—
Dimethl sulfone	—	*p*-Chloroamphetamine HCl	—
Diphenhydramine HCl	Bright blue ppt	Pentazocine HCl	—
Ephedrine HCl	—	Pentermine HCl	—
Ethylmorphine	—	Phencylcidine HCl	Bright blue ppt
Fenfluramine HCl	—	Phendimetrazine	Pink solution
Fentanyl citrate	Light blue ppt	Phenobarbital	—
Flunitrazepam	—	Phenylepropanolamine HCl	—
Flurazepam 2HCl	Green ppt	Procainamide HCl	—
γ-butyrolactone (GBL)	Pink solution	Procaine HCl	—
GHB, Na	—	Pseudoephedrine HCl	—
Guaifenesin	—	Quazepam	—
Heroin HCl	—	Secobarbital, Na	—
Hydrocodone bitartrate	Blue ppt	Sibutramine HCl	—
Hydromorphone HCl	—	Sodium bitartrate	Pink solution
Ibogaine HCl	—	Sodium carbonate	Light blue ppt
Ketamine free base	—	Sodium sulfate	—
Ketamine HCl	Purple/lavender ppt	Soluble starch	—
Lactose	—	Temazepam HCl	—
Levopropoxyphene napsylate	—	Triazolam	—
Levorphanol tartrate	Light blue ppt	Zolpidem tartrate	Blue ppt

—, a blue-green precipitate.

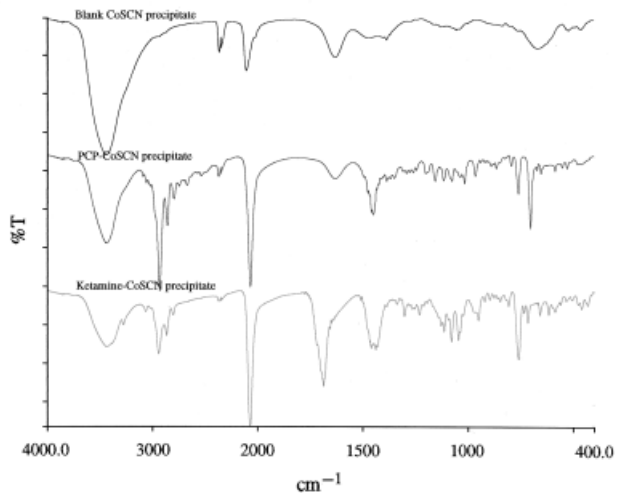

FIG. 1—*FTIR spectra of ketamine, PCP, and blank precipitates.*

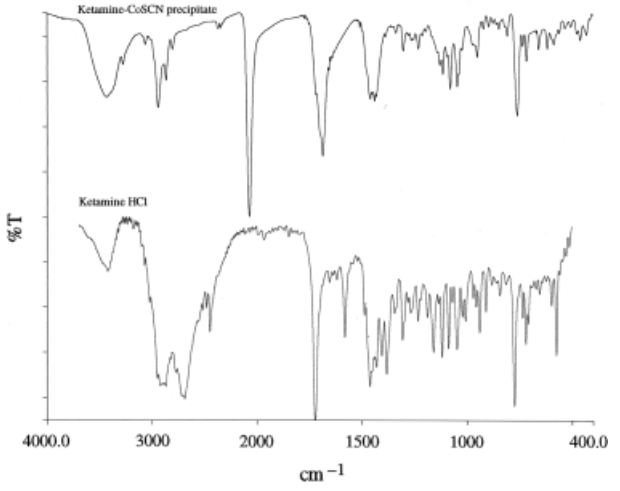

FIG. 2—*FTIR spectra of the ketamine–cobalt thiocyanate (CoSCN) precipitate and Ketamine HCl.*

The necessity of the thiocyanate ion was confirmed when FTIR analysis of the precipitate revealed a strong cyanate peak at *c*. 2066 cm^{-1} (Fig. 2). Further confirmation of the necessity of the thiocyanate ion occurred when substitution of the CoSCN solution with a cobalt nitrate solution resulted in a light blue precipitate. The necessity of the hydroxide ion was confirmed when substituting neutral or acidic solutions for the 0.1 N NaOH solution did not give a lavender to purple precipitate.

The necessity of the chloride ion was confirmed in several ways. Ketamine-free base was isolated and dried. A portion of the dried material was placed in a spot well following the standard modified CoSCN procedure. Instead of a purple to lavender precipitate, a bright blue precipitate formed. Replacing the deionized water with a 25% HCl solution resulted in a faint purple to lavender precipitate. Converting the ketamine free base to ketamine sulfate also did not result in the purple to lavender precipitate expected with ketamine hydrochloride.

Sensitivity

As the color change is attributed to the formation of a complex, the test was not expected to be extremely sensitive. Stock solutions of reference ketamine hydrochloride were prepared in deionized water at concentrations of 100, 50, 25, 10, 5, 1, 0.5, 0.25, and 0.1 mg/mL, and tested using the "General Test Procedure for Liquid Samples." Purple to lavender precipitates were observed for the 100, 50, 25, and 10 mg/mL solutions. An inconsistent color change was originally observed for the 5 mg/mL solution. However, when the sample volume was increased from one drop to $\sim$ 0.25 mL, a lavender precipitate was observed. Only blue–green precipitates for 1, 0.5, 0.25, and 0.1 mg/mL solutions were observed for both one drop and $\sim$ 0.25 mL sample sizes. The results indicate a detection limit of $\sim$ 5 mg/mL or $\sim$ 1.25 mg. This is consistent with the reported sensitivity of ketamine with the Janovsky reagent. Commercial ketamine hydrochloride products have typical concentrations of 50–100 mg/mL.

Ruggedness

Varying the concentration of the hydroxide solution had varying effects on the resultant colored precipitate. Sodium hydroxide solutions of 2, 1.0, 0.5, 0.25, 0.05, 0.025, and 0.01 N were examined in addition to the 0.1 N solution. The 0.01 N NaOH solution resulted in a clear pink solution. The 2, 1.0, and 0.5 N solutions resulted in a blue precipitate with the precipitate of the 2 and 1.0 N solutions gradually changing to a dark yellow.

A purple to lavender precipitate was obtained with the 0.25, 0.1, 0.05, and 0.025 N solutions. However, as the normality of the solution increased, the amount of sample necessary to achieve the precipitate also increased. Although the 0.05 and 0.025 N solutions required less sample to obtain a purple to lavender precipitate, blank runs of these solutions gave pink solutions with a small amount of blue–green precipitate, which could be confused with a weak positive result (e.g., a faint purple lavender precipitate in a clear solution compared with a pink solution). Although the 0.1 N solution required slightly more sample for the necessary precipitate, the blue–green precipitate for blanks gave a stronger contrast between positive and negative results.

Substituting the 0.1 N NaOH solution with a 0.1 N KOH solution, a 0.1 N NH_4OH solution, or a saturated sodium bicarbonate solution all resulted in lavender to purple precipitates. A 2% bicarbonate solution did not yield the expected colored precipitate.

Substituting tap water for the deionized water did not adversely affect color formation. Dissolving ketamine hydrochloride in solvents other than deionized water did not adversely affect the test. Solutions of ketamine in methanol, ethanol, tap water, and a saline solution all gave the indicative lavender to purple precipitate. When tested as blanks, all of these same solutions gave a blue–green precipitate.

Conclusion

A modified CoSCN presumptive color test for ketamine hydrochloride was presented. The test has good specificity. Of all the compounds tested in this study, only ketamine hydrochloride gave a lavender to purple precipitate. The test is sufficiently sensitive enough for analysis of commercial ketamine hydrochloride products.

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