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Duquenois levine reagent test

Presumptive tests for cannabis products are used to test for the presence (or otherwise) of phenolic cannabinoids. The principle one that is used is the Duquenois-Levine test. An alternative which is available and can also be used is the Corinth IV salt test. Both involve a reaction between the cannabinoids and a test reagent to form coloured products. Positive and negative control tests should also be carried out. [Pg.58] The Duquenois-Levine Test In this, three reagents are required (see Appendix I), with the reaction being carried out in small test-tubes. The three reagents are... [Pg.58] Tests for Marijuana The Duquenois reagent (Duquenois or Duquenois-Levine test) involves a condensation reaction leading to creation of a purple chro-mophore (Figure 7.19) with the active ingredient tetrahydrocannabinol. Marijuana analysis frequently includes TLC using Fast Blue BB salt (or a similar... [Pg.287] The presumptive color test for marijuana is the Duquenois-Levine test. A portion of the sample is extracted with petroleum ether and transferred to a test tube. The Duquenois reagent is added, followed by hydrochloric acid. A bluish-purple color is indicative of cannabinoids. Several drops of chloroform are added to the test tube, and it is shaken imtil the purple color drops into the chloroform layer. Depending on the concentration of cannabinoids, this transfer can be complete, leaving the top layer in the test tube nearly clear. Potential false positives occur in the presence of some coffees and other oils, but, as with most color-based presumptive tests, an experienced analyst will often recognize a false positive as being subtly different from a true positive. See the color insert for photos of the Duquenois test. [Pg.305] In some laboratories, the analysis stops with the Duquenois-Levine test, but many add TLC with standards as the final step, using the dye Fast Blue B (which is carcinogenic), or Fast Blue BB or the Duquenois reagent as a developer. Fast Blue B gives the constituents distinctive colors A -THC turns red, CBN purple, and CBD orange. The combination of microscopy, the Duquenois-Levine test, and TLC with standards is considered conclusive identification for marijuana. Quantitation of THC is currently not required in the United States. Derivatization techniques can be used for GC, but HPLC is better suited to such analyses. [Pg.305] Name the functional group(s) in THC. What presumptive color or crystal tests could be useful, aside from the Duquenois-Levine test Why are crystal tests of limited use with plant extracts ... [Pg.316] Resorcinol gives a false positive with the Duquenois-Levine test. Why ... [Pg.316] Marijuana (herbal cannabis) is the most common illegal dmg in the world. Seizures have increased globally with the Americas accounting for the highest share (1). U.S. seizures of marijuana alone exceed 1 million kilograms each year (6). Evidence submitted to forensic laboratories is screened for marijuana by microscopic inspection and simple chemical tests such as the Duquenois-Levine test. Presumptive positive results are confirmed by GC/MS to positively identify cannabinoids, including... [Pg.261] Seized samples of marijuana are analyzed in the laboratory using a color test, thin-layer chromatography, and a microscopic test. The Duquenois-Levine color test, although not specific for marijuana, is often used. Using the microscope one can see on the upper side of the marijuana leaf characteristic bear claw -shaped cystolithic hairs, which contain calcium carbonate. [Pg.60] Duquenois-Levine A presumptive test for THC, the active ingredient in marijuana. [Pg.619] A Figure 4 The full Duquenois-Levine color test. Note the purple compound that has extracted into the lower chloroform layer. [Pg.695] Cannabis usually comes in three forms (1) cannabis (marijuana), (2) cannabis resin (hashish), and (3) extracts of cannabis resin (hashish oil). Most laboratories use a color test (modified Duquenois-Levine), a morphological examination... [Pg.897] Share — copy and redistribute the material in any medium or format for any purpose, even commercially. Adapt — remix, transform, and build upon the material for any purpose, even commercially. The licensor cannot revoke these freedoms as long as you follow the license terms. Attribution — You must give appropriate credit , provide a link to the license, and indicate if changes were made . You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use. ShareAlike — If you remix, transform, or build upon the material, you must distribute your contributions under the same license as the original. No additional restrictions — You may not apply legal terms or technological measures that legally restrict others from doing anything the license permits. You do not have to comply with the license for elements of the material in the public domain or where your use is permitted by an applicable exception or limitation . No warranties are given. The license may not give you all of the permissions necessary for your intended use. For example, other rights such as publicity, privacy, or moral rights may limit how you use the material. Technical Note Specificity of the Duquenois-Levine and Cobalt Thiocyanate Tests Substituting Methylene Chloride or Butyl Chloride for Chloroform Amanda J. Hanson Wisconsin State Crime Laboratory Madison 4626 University Avenue Madison, WI 53705 2156 [email: hansonaj -at- doj.state.wi.us] ABSTRACT: The use of alternative solvents in the Duquenois-Levine and Cobalt Thiocyanate tests were explored due to substandard results with recently purchased lots of chloroform. Methylene chloride provided satisfactory results when substituted for chloroform in both tests. Butyl chloride provided satisfactory results in the Duquenois-Levine test. KEYWORDS: Duquenois-Levine, Cobalt Thiocyanate, Marijuana, Cocaine, Chloroform, Methylene Chloride, n-Butyl Chloride Introduction The Rapid Modified Duquenois-Levine test and Cobalt Thiocyanate test (Scott test) are proven screening tests for the presence of marijuana and cocaine, respectively. The organic solvent traditionally used in these tests is chloroform. However, chloroform recently purchased by this laboratory produced little or no color change when performing the Duquenois-Levine and Cobalt Thiocyanate tests. Shortly after opening, this chloroform became yellow to green in color, at which point it was unsuitable to perform these tests. According to the manufacturer, this unusual decomposition of the chloroform was due to insufficient amounts of preservatives. This experience led to the investigation of using alternative organic solvents, specifically methylene chloride and n-butyl chloride, in the Duquenois-Levine and Cobalt Thiocyanate tests. Experimental Reagents and Solvents Hydrochloric acid, methylene chloride, and n-butyl chloride were obtained from Fisher Scientific. Acceptable quality chloroform was obtained from OmniSolv. The Duquenois reagent was prepared by adding 10 grams of vanillin and 5 milliliters of acetaldehyde to 500 milliliters of ethanol. The vanillin, acetaldehyde, and ethanol were obtained from Kodak, EM Science, and Fisher Scientific, respectively. The cobalt thiocyanate reagent was prepared by dissolving ten grams of cobalt (II) thiocyanate in a mixture of 490 milliliters of distilled water and 500 milliliters of glycerin. The cobalt (II) thiocyanate and glycerin were obtained from Aldrich Chemical and Fisher Scientific, respectively. Procedures The Duquenois-Levine test was performed on 17 different substances using chloroform, methylene chloride, or butyl chloride as the organic solvent. The test was performed by placing approximately 10 to 20 milligrams of a target substance in a glass test tube, then 10 drops of the Duquenois reagent. After shaking, 10 drops of concentrated hydrochloric acid were added, and the tube was again shaken. Any color that resulted after the hydrochloric acid step was recorded. Twenty drops of chloroform were then added, and the tube was vortexed, then allowed to settle and separate into two layers. Any color that transferred into the organic layer was recorded (Table 1). This procedure was repeated for each target substance by substituting methylene chloride or butyl chloride for chloroform. The cobalt thiocyanate test was performed on 14 different substances using chloroform, methylene chloride, or butyl chloride. The test was performed by placing approximately 2 to 4 milligrams of a target substance in a glass test tube, then 5 drops of cobalt thiocyanate reagent. After shaking,1 or 2 drops of concentrated hydrochloric acid were added, and the tube was again shaken. Ten drops of chloroform were then added, and the tube was vortexed, then allowed to settle and separate into two layers. The final color of the chloroform (organic) layer was recorded (Table 2). This procedure was repeated for each target substance by substituting methylene chloride or butyl chloride for chloroform. Results and Discussion The results for the Duquenois-Levine test using either methylene chloride and butyl chloride were consistent with results obtained using chloroform. The marijuana became purple with the addition of the Duquenois reagent and hydrochloric acid. Upon addition of the organic solvent, the purple color transferred to the organic layer, indicating a positive test for cannabinoids. The color was consistent in all tests involving marijuana, regardless of the solvent used. None of the remaining 16 substances tested gave the characteristic purple color in the organic solvent layer. Similarly, the results of the Cobalt Thiocyanate test were equivalent whether chloroform or methylene chloride was used. However, the results for the butyl chloride were mixed. Addition of the cobalt thiocyanate reagent to cocaine hydrochloride resulted in the surface of the particles turning a bright blue (faint blue for cocaine base). The solution changed back to pink upon adding one or two drops of hydrochloric acid and mixing. Addition of 10 drops of chloroform, vortexing, and allowing the solution to settle resulted in a blue organic layer for both cocaine hydrochloride and cocaine base. The test had similar results when methylene chloride was substituted for chloroform. In the case of butyl chloride, however, the organic layer stayed clear, giving an inconclusive test. Diphenhydramine and lidocaine also gave blue organic layers with either chloroform and methylene chloride. These compounds are known false positives for cocaine. However, in the case of butyl chloride, the organic layers were clear for diphenhydramine and white for lidocaine. The other ten materials had consistent negative test results for all three organic solvents. Conclusions Methylene chloride may be substituted for chloroform in both the Rapid Modified Duquenois-Levine test and Cobalt Thiocyanate test. Similarly, butyl chloride may be substituted for chloroform in the Duquenois Levine test. However, butyl chloride was not a reliable substitute solvent for use in the Cobalt Thiocyanate test. Methylene chloride also works well as an extraction solvent in place of chloroform. [Tables 1 and 2 Follow.] Table 1. Duquenois-Levine Test Results Chloroform Methylene Chloride Butyl Chloride Material aqueous/organic aqueous/organic aqueous/organic Allspice brown/clear brown/clear brown/clear Celery Flakes yellow/clear yellow/clear yellow/clear Chamomile yellow/clear yellow/clear yellow/clear Chamomile Tea yellow/clear green/clear green/clear Coffee brown/clear brown/clear brown/clear Dill Seed yellow/clear yellow/clear yellow/clear Hops yellow/clear yellow/clear yellow/clear Ginger orange/orange orange/orange orange/clear Ginseng brown/brown green/clear green/clear Marijuana purple/purple purple/purple purple/purple Marjoram yellow/clear green/clear green/clear Mint green/clear green/clear green/clear Sage yellow/clear yellow/clear yellow/clear Salvia Divinorum green/clear brown/clear green/clear Thyme yellow/clear green/clear green/clear Tobacco brown/clear brown/clear brown/clear White Pepper orange/yellow orange/yellow orange/yellow Table 2. Cobalt Thiocyanate Test Results Chloroform Methylene Chloride Butyl Chloride Material organic layer organic layer organic layer Benzocaine clear clear clear Cocaine blue blue clear Cocaine Base blue blue clear Dextrose clear clear clear Diphenhydramine blue blue clear Heroin clear clear clear Inositol clear clear clear Lidocaine blue blue white Methamphetamine clear clear clear MDMA clear clear clear Morphine clear clear clear Procaine clear clear clear Soap clear clear clear Sodium Bicarbonate clear clear clear Cite Save Email this content Copy this link, or click below to email it to a friend Email this content or copy the link directly: As a library, NLM provides access to scientific literature. Inclusion in an NLM database does not imply endorsement of, or agreement with, the contents by NLM or the National Institutes of Health. Learn more: PMC Disclaimer | PMC Copyright Notice . 2015 Mar 6;33(2):175–194. doi: 10.1007/s11419-015-0270-0 A number of N-alkyl indole or indazole-3-carbonyl analogs, with modified chemical structures, are distributed throughout the world as synthetic cannabinoids. Like synthetic cannabinoids, cathinone analogs are also abused and cause serious problems worldwide. Acute deaths caused by overdoses of these drugs have been reported. Various analytical methods that can cope with the rapid changes in chemical structures are required for routine analysis and screening of these drugs in seized and biological materials for forensic and clinical purposes. Although many chromatographic methods to analyze each drug have been published, there are only a few articles summarizing these analytical methods. This review presents the various colorimetric detections, immunochemical assays, gas chromatographic–mass spectrometric methods, and liquid chromatographic–mass spectrometric methods proposed for the analysis of synthetic cannabinoids and cathinones. Keywords: Synthetic cannabinoids, Cannabimimetics, Cathinones, GC–MS-MS, LC–MS-MS, Analytical methods Currently, many illegal drugs are abused worldwide, with serious social problems arising as a consequence. Although various stimulants and narcotics have been in use to date, new drugs targeting cannabinoid receptors have been abused since their existence in herbal mixtures was disclosed in 2008 [1]. HU-210, a synthetic classical cannabinoid, and cyclohexylphenols were commonly used as recreational drugs, but mainstream use has since changed to N-alkyl indole-3-carbonyl derivatives, such as drugs of the JWH and AM series (Fig. 1), because their activities are stronger than those of the conventional cannabinoids. These compounds are called cannabimimetics or synthetic cannabinoids and can be purchased as “spice” or “K2” in the drug market or via the Internet. Cathinones, also known as “bath salts” or “plant food,” are psychoactive drugs and are also abused as recreational drugs. The parent compound, cathinone, is a well-known stimulant, and can be isolated from the khat plant or produced by synthetic means. Cathinone analogs with high selectivity and strong activity for serotonin receptors and monoamine transporters have been distributed in the drug market (Fig. 2). The prevalence of cannabinoid and cathinone abuse in many countries has been reviewed elsewhere [2–7]. Structures of synthetic cannabinoids Structures of cathinonesAlthough the same substances are distributed throughout the world, the times at which they are abused tend to vary depending on whether the substances are controlled by local laws. As shown in the reviews [2–7], new analogs appear in the drug market just after the preceding drug comes under regulation. Although many such substances are controlled in countries throughout the world, the regulations are usually limited by the structures of the drugs. Therefore, when the structure of a side chain or substitution is slightly different from that of the regulated drug, the analog is regarded as being beyond the scope of the regulation. These emerging drugs always show psychoactive actions because their chemical structures are similar to those of the drugs being controlled. However, the detailed pharmacological activities of these analogs are not known, which makes access easy and use of these drugs very dangerous to human health. Although many researchers have focused on the development of detection methods, only a few analytical reviews that summarize the systematic identification and quantification techniques for these drugs have appeared [8–10]. In this review, we summarize the various techniques for the detection of synthetic cannabinoids and cathinones that have been published up to 2014, including colorimetric, immunochemical, and chromatographic methods. The Duquenois–Levine color test, which is used to identify classical cannabinoids such as Δ9-tetrahydrocannabinol, is negative for the synthetic cannabimimetics. The van Urk color test, which is used to identify indole-containing drugs of abuse, is also negative for these compounds. The use of 2,4-dinitrophenylhydrazine, which reacts with a keto moiety, is capable of reacting with synthetic cannabimimetics, such as the naphthoylindole, phenylacetylindole, benzoylindole, and cyclopropylindole classes, either in powder form or adsorbed onto plant material, and a positive test solution turns from yellow to orange. Although the LOD concentration was not detailed in the article, the solution tested contained at least 10 mg of cannabimimetic powder suspended in methanol (1 ml) [11]. The Marquis reagent, which reacts with all nitrogen-containing drugs, is positive for cyclohexylphenols and the JWH series. Although Dragendorff reagent is also positive for the JWH series, its LOD concentration is higher than that of Marquis reagent. Fast blue BB reacts with cyclohexylphenols, and the LOD concentration is not lower than that of Marquis reagent [12]. Iodoplatinate is also used as a detection reagent after TLC [13]. Although it is possible to detect synthetic cannabinoids with each reagent in these screening tests, it is difficult to detect small amounts or mixtures of synthetic cannabinoids. ELISAs developed in-house could be calibrated at 5 ng/ml with the 5-OH and 4-OH metabolites of JWH-018 and JWH-250, respectively, and evaluated for the detection of synthetic cannabinoids in urine [14]. Recently, some commercially available immunoassay kits, such as DrugCheck K2/Spice Test, DrugSmart Cassette, and RapiCard InstaTest, have been developed for the detection of these drugs in urine. These devices are more useful than the colorimetric methods, because they do not require special reagents or tools, and the results are obtained easily and quickly. The devices also can detect older types of synthetic cannabinoids, such as JWH-018 or JWH-073, but, unfortunately, new designer drugs such as QUPIC and AB-CHMINACA cannot be detected. Typical mass spectra of synthetic cannabinoids are shown in Fig. 3. Molecular (M+) and/or fragment ions observed by full scan data acquisition of GC–MS reflect the structures of the synthetic cannabinoids [13, 15, 16]. As shown in Fig. 4, the fragmentation pathways of naphthoylindoles have been well studied for the identification of synthetic cannabinoids by GC–MS [12, 15]. Therefore, the identification of synthetic cannabinoids is facilitated by comparison of the spectra with commercial and open databases. Typical mass spectra of synthetic cannabinoids obtained by GC–MS. a JWH-018, b RCS-4, c JWH-250, d AM-1220, e THJ-018, f APICA, g NNEI, h ADBICA, i QUPIC (PB-22), j ADB-PINACA, k AB-CHMINACA Probable fragmentation pathways of synthetic cannabinoids by electrospray ionization and electron ionization (modified from references [12, 31])In naphthoylindoles, the carbonyl group fragment ions, which are caused by α-cleavage of the alkylamino group of the indole, are typically observed. In addition, [M−17]+ is certainly observed in naphthoylindoles. For example, fragment ions at m/z 284 and 214 are observed in JWH-018, corresponding to those of the indole moiety caused by α-cleavage of the N-pentyl of indole and naphthoyl. Fragment ions at m/z 127 and 155 are observed in JWH-018, corresponding to the naphthalene group caused by the α-cleavage of the carbonyl group. Moreover, ions at m/z 324 are observed as [M−17]+ (Fig. 3a). Like naphthoylindoles, fragment ions caused by α-cleavage of the alkylamino group of the indole and carbonyl groups are shown, although [M−17]+ is not observed for benzoylindoles. For example, fragment ions at m/z 264 and 214 are observed for RCS-4, caused by α-cleavage of N-pentyl of the indole and 4-methoxybenzoyl. The ions at m/z 127 and 155, which are caused by naphthyl and naphthoyl moieties of naphthoylindoles (Fig. 3a), and the ion at m/z 135 caused by the 4-methoxybenzoyl moiety (Fig. 3b) are useful as precursor ions for identification of these drugs by GC–MS-MS. On the other hand, the methylpiperidine moiety is bound to the nitrogen of the indole, and the ion at m/z 98 is observed as the base peak (Fig. 3d). Unlike naphthoyl and benzoyl indoles, the base peak of the fragment ion caused by the N-alkylindole 3-carbonyl moiety for phenylacetyl (Fig. 3c), cyclopropyl, or adamantyl (Fig. 3f) indoles, is only shown in each full scan spectrum. Analogs, in which the indole skeleton is changed to an indazole, such as THJ-018, have also appeared on the market. In these analogs, molecular and N-dealkylated ions are typically observed in the spectrum (Fig. 3e). Recently, amide- or ester-type analogs bonded with an N-alkylindole or N-alkylindazole 3-carbonyl moiety have appeared on the market [17, 18]. In these analogs, the abundance of the molecular ion is low, and the fragment ion caused by the indoyl (or indazoyl) moiety is observed as a base peak (Fig. 3f–k). Although the fragment ion caused by elimination of the terminal CO–NH2 is lower than that of the cleavage of the amide moiety in indole analogs, such as ADBICA (Fig. 3h) [19], the fragment ion caused by elimination of terminal CO–NH2 is as intense as that of the cleavage of the amide moiety in indazole analogs, such as ADB-PINACA and AB-CHMINACA (Fig. 3j, k) [20]. The substitution of the indole skeleton with the indazole moiety, such as in THJ-018 and THJ-2201 [21], has also been observed in these analogs. In these analogs, molecular and N-dealkylated ions are typically observed in the spectrum. For example, in the simultaneous analysis of synthetic cannabinoid species, 10 mg of ground powder of the dried leaves was extracted with 10 ml of methanol under ultrasonication for 10 min. The extracts were centrifuged for 5 min at 3,000 rpm, and the supernatants were filtered and used for GC–MS analysis. The LODs were 0.5–1.0 mg/l, and linearity was obtained at concentrations up to 100 mg/l [16]. In another article [22], herbal samples (approximately 50 mg) were put into 10-ml headspace vials, and the vials were capped with 20-mm magnetic crimp seal caps with PTFE/silicone septa. The samples were incubated at 200 °C with pulse-agitation at 250 rpm. A StableFlex carboxen/polydimethylsiloxane fiber was inserted into the headspace for 5 min for extraction. The fiber was then injected into the GC inlet for 15 min to desorb the analytes. The LOD of synthetic cannabinoid in the samples was at least 20 μg. The tentative identification of synthetic cannabinoids appears easy, but similar mass spectra are sometimes obtained by GC–MS because regio- and ring-substituted analogs are still distributed on the market. The misidentification of these analogs arises when using only the information from the mass spectra. When tandem and high-resolution MS are used to identify the conformational isomers or regioisomers, such misidentification does not occur [23–28]. Moreover, identification of cyclopropyl or ester analogs, such as UR-144 or QUPIC, is usually not possible because cyclopropyl analogs are heat-unstable and are easily degraded in the injection port of the GC instrument [29, 30]. Many research groups have used LC–MS-MS for determination of synthetic cannabinoids in herbs and biological samples, and some have studied the fragmentation of synthetic cannabinoids in detail [15, 31]. The probable fragmentation pathways are shown in Fig. 4. Because the protonated molecular ion is only observed by LC–MS, and the information acquired by LC–MS is lesser than that for GC–MS, it is necessary to obtain other data that reflect the chemical structures by LC–MS-MS or TOFMS. Fragment ions are observed by product ion scanning when the protonated molecular ion is used as the precursor ion. In naphthoylindole, ions at m/z 127 and 155 are generated by naphthyl and naphthoyl moieties. However, information about the indole moiety tends to be not revealed by LC–MS-MS. On the other hand, the N-alkyl moiety of a synthetic cannabinoid is mainly modified for excretion into urine as a metabolite. Therefore, LC–MS-MS is a useful methodology to search for metabolites of synthetic cannabinoids in urine. Recently, packages containing mixtures of multiple synthetic cannabinoids have been sold commercially, even though the package ingredients have been largely unknown to both sellers and buyers. In this aspect, the LC–MS-MS screening method is helpful in some estimation of the ingredients. Kneisel and Auwärter [32] demonstrated the simultaneous detection of 30 synthetic cannabinoids in serum; the LODs and LOQs were 0.01–2.0 and 0.1–2.0 ng/ml, respectively. There are many applications for analysis of synthetic cannabinoids in urine, hair, and oral fluids [33–37]. The typical published methods for analysis of synthetic cannabinoids in biological materials are summarized in Table 1 [38–54]. Simple LLE is usually used for the extraction of synthetic cannabinoids from biological materials because of the high hydrophobicity of the drugs. The chromatographic conditions are generally simple and do not require a special technique; octadecyl-type columns were used as analytical columns and analyses were performed in gradient mode. LC–MS or LC–MS-MS conditions for synthetic cannabinoids in biological materials Target(s) Sample(s) Purification(s) Column(s) Mobile phase LOD (ng/ml) Linear range (ng/ml) Reference(s) JWH-018 Serum LLE Luna C18 (2) (150 mm, 2 mm ID, 5 μm) (Phenomenex) 10 mM ammonium acetate (0.1 % acetic acid, pH 3.2), methanol 0.07 0.21–20 [38] JWH-018, JWH-073, JWH-019, JWH-250 Blood LLE Acquity UPLC HSS T3 (100 mm, 2.1 mm ID, 1.8 μm) (Waters) 1 % formic acid, methanol (1 % formic acid) 0.006–0.016 0.1–20 [39, 40] Aminoalkylindoles, methanandamide Serum LLE Luna phenyl hexyl (50 mm, 2 mm ID, 5 μm) (Phenomenex) 2 mM ammonium formate (0.2 % formic acid), methanol 0.1 0.1–2, 0.3–2 (methanandamide) [41] JWH-018, JWH-073, metabolites Urine Dilution (hydrolysis) Zorbax Eclipse XDB-C18 (150 mm, 4.6 mm ID, 5 μm) (Agilent) 0.1 % formic acid, acetonitrile (0.1 % formic acid)