Identification of drugs in solid state using spectral methods

Immunochemical diagnostic tests

Spectral methods in forensic chemistry represent one of the major and dynamically developing methods. They allow objective identification of an unknown substance based on different physico-chemical principles. One of the methods in forensic laboratories is commonly used infrared spectroscopy (IR). Infrared radiation is an electromagnetic radiation in the range of about 800 nm to about 1000 micrometers. Unlike spectrometry in the visible or UV region, where the principle of the absorption of radiation are based on the valence electron excitation to a virtual level, IR radiation operates with lower energies. When molecules absorb IR it leads to changes in their vibrational state, which are characteristic of a specific wave numbers (IR spectroscopy uses reciprocal value of the wavelength, expressed in cm\(^{-1}\)). Atoms in molecules vibrate at specific frequencies. If IR radiation frequency is identical to the frequency of vibration of the atom, there will be absorption of this radiation. Many functional groups are characterized by their wave numbers, so in addition to the identification of unknown substances some basic information on its possible structure may be obtained. The common method of the infrared spectroscopy is a transmission measurement of IR spectra, in which there is absorption of IR radiation of the compound. The result is called an IR spectrum, providing information of some of the functional groups and in the range of about 200 - 1500 cm\(^{-1}\) a "fingerprint" which is poorly interpretable and can be used to identify unknown substances. IR advantage is the possibility of measuring samples as a liquid (e.g. a thin film) and the solid state (KBr or nujol technique). The huge disadvantage is the fact that water (as the most common solvent) exhibit strong absorption of IR radiation and samples must be dried properly prior to the analysis.

Because of the fact that there are thousands of organic substances incl. the controlled substances (addictive substances, drugs, prodrugs, etc.) preliminary qualitative methods must be done before the instrumental analysis. Besides the basic physico-chemical description (appearance, color, crystalline structure, solubility, fluorescence, odor, pH, etc.) some preliminary reactions can confirm or exclude the presence of an unknown substance in the sample.

Chemical reactions leading to complete or preliminary identification of a suspected substance (addictive drugs, psychotropic drugs or other drugs) represent a wide variety of methods based on simple tests, which ideally eliminates or directly confirms the presence of the test compound in the sample. In many cases, these conventional chemical reactions used in the qualitative analysis of organic compounds are based on more or less selective reactions. There are many processes and methods that can confirm the analytes in the sample. Generally, for practical applications it is advisable to find the most specific test held under normal conditions (room temperature, available solvents, material, etc.).

Preliminary test is usually performed with several reagents and sample directly in the solid state (e.g. crystalline methamphetamine) or after its conversion into a solution in an appropriate solvent (typically water or polar organic solvents such as methanol or ethanol).
Below is an overview of the most commonly used chemical reagents that are used in practice to detect a broad range of suspicious substances.

1.) Marquis reaction

It is one of the most common reaction for preliminary testing of suspected substances. It is used in analyzing the drug but also to identify the alkaloids or commonly used drugs, which significantly reduces its selectivity. However, its relative simplicity and especially the combination with additional tests (see below) can lead to confirmation of suspected drug in the sample. It is used primarily when testing ecstasy (MDMA), drugs derived from amphetamines (phenylethylamine derivatives) and opioids. It is possible to distinguish the commonly abused drug - MDMA and methamphetamine which is normally very complicated.

The reagent is used a mixture of concentrated sulfuric acid and formaldehyde (100 ml of 98% sulfuric acid is mixed with 5 mL of 38-40% aqueous solution of formaldehyde). The reaction mechanism is quite complex, it can be said that it is based on the polymerization of molecules of the test compound and formaldehyde in the acidic environment where charged oxonium or carbonium compounds (Fig. 1) are formed. An example of Marquis reagent reaction with amphetamine is shown in Fig. 2.

![Fig. 1: Oxonium and carbonium ions](image1)

![Fig. 2: Marquis reaction of amphetamine (R = H) and methamphetamine (R = CH₃)](image2)
Marquis reagent is recommended especially for opiates, which provides the dark purple or reddish products. Frequent substitute or "thinner" of drugs based on opiates (heroin, morphine, codeine, etc.), dextromethorphan (DXM) provides deep black precipitate. This can be easily distinguished from DXM of other opiates or opioids. With amphetamine-type substances products of red or brown color are formed, which darkens over time. If the molecule contains the methylendioxide group (e.g. MDMA), then the violet color is formed. However, Marquis reagent reacts with many conventional chemicals. These substances include sugars (sucrose, glucose), having a characteristic dark brown color. When used with acetylsalicylic acid (aspirin) it provides a red or orange product (in about 2-3 min) and can lead to a false positive conclusion on the presence of amphetamines.

2.) Simon’s reagent

Simon’s reagent is a relatively selective reagent for aromatic secondary amines (its modification called Robadop’s reagent is selective for primary amines). A positive reaction will be reflected as a light or dark blue color, at lower concentration of test compound often after several minutes. It is a secondary amine reacting with acetaldehyde to form an enamine, which is subsequently reacted with sodium nitroprusside to give an immonium salt which is subsequently hydrolyzed to Simon-Awe complex (Fig. 3). It is characterized by its blue color.

![Chemical reaction diagram]

Fig. 3: Simon’s reaction with methamphetamine giving a blue Simon-Awe complex with sodium nitroprusside

3.) Chen-Kao reagent
Some substances which are regarded as drugs or their precursors do not react with any conventional testing reagent. A typical example is ephedrine and its derivatives (incl. its enantiomers). Although ephedrine is also a secondary amine, containing a hydroxyl group in its molecule thus gives a negative reaction with the Simon's reagent. Because of the fact that ephedrine and its derivatives (pseudoephedrine, chloroefedrin, chloropseudoefedrin etc.) are important precursors in the synthesis of methamphetamine (Fig. 4), a reagent for this type of compounds is very important.

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\text{A)} \quad \text{Ephedrine} \quad \text{SOCl}_2 \quad \text{Cl} \quad \text{Pd/H}_2 \quad \text{methamphetamine}
\]

\[
\text{B)} \quad \text{ephedin}
\]

Fig. 4 – Methamphetamine synthesis from ephedrine (chloroephedrine, A) and a stereoselective synthesis from (+) or (-) pseudoephedrine using the reduction by hydriodic acid catalyzed by phosphorus.

The Chen-Kao reaction utilizes formation of violet or blue complex with copper ions (Fig. 5) and is more or less selective for phenylalkylamines with vicinal hydroxy and amino groups. Reaction with amphetamine-type substances does not occur. The reaction may also take place with norephedrine or ephedrine chloroderivatives when the coloring is dark blue. The resulting complex can be extracted to non-polar solvent such as diethyl ether or n-butanol.
4.) The detection of THC using "Fast" diazonium salts series

The so-called Fast salts are diazonium compounds used in analytical chemistry and biochemistry, especially as dyes and derivatizing agents. Several compounds from this series, however, allow very selective detection of THC in marijuana and its products. One of the most used is the Fast Blue B (or BB, Fig. 6).

This compound reacts in basic medium with two molecules of THC to producing characteristically colored compound (Fig. 7). Color and reaction speed depends on the type of dye, in practice most often used Fast Blue B (positive reaction is red) and then Fast Garnet GC (resulting color is orange), Fast Blue RR (resulting color is pink) or Fast Corinth V (resulting color is purple). The most common method of detection is to perform extraction of the suspected material (e.g. dried marijuana or hashish) to ethanol followed by addition of the selected Fast salt (usually mixed with anhydrous sodium sulfate in the ratio of 1: 100). The basic environment is reached by adding a few drops of 2 to 5% sodium carbonate solution. If the color of the extract makes it impossible to observe the color change the colored product can be extracted tonon-polar solvent (e.g. chloroform).
Immunochemical methods are based on the specific reaction of the antigen (analyte) with the antibody. The condition is the existence of specific antibody that is sensitive to the presence of analyte in the sample. These methods work on the principle of competition between the analyte and the labeled analyte (usually radioactively, fluorescently, enzymatically etc.) present on the antibody. Analyte detection is then based on its ability to displace the labeled analyte from binding to the antibody. It is technically easy methods that are often insufficiently specific and therefore it is necessary to confirm a positive result by another objective analytical method. At present there are specialized laboratories with automatic Immunoassay Analysator which are able to quickly analyze particularly biological samples. For a quick orientation analysis or confirmation, there are also single immunochromatographic assays that operate on a similar principle with visual evaluation result (Fig. 8).

Immunochemical tests work on the principle of the chromatographic capillary action of the sample like paper chromatography or TLC. The test sample migrates into an area where there is a labeled antibody (in this case usually color). If the test sample contains the analytes (or its content is lower than the detection limit of the assay), there is no antibody binding, and
in certain areas of the test antibody binds to the so-called drug protein (which is immobilized in the test). A negative result is then reflected as two visible color zones (see figure). Otherwise, the present analyte inhibits binding of the antibody to the drug and the protein itself forms a complex with it. It appears as a missing color in the test zone. To verify the functionality of the test the second (control) zone must be always positive regardless of the presence of the substance sought. Immunochemical tests can be highly specific (e.g. test for THC, respectively. Metabolite thereof), on the other hand, may react non-selectively with a variety of similar substances including metabolites. This fact must be taken into account when assessing the results and immunochemical test should always serve rather as a fast and approximate method to confirm or exclude searching drugs or compounds.