Drugs of Abuse: Analyses and Ingested Agents That Can Induce Interference or Cross-Reactivity

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Abstract
Drug abuse is rampant in society having a detrimental effect towards public safety and productivity in industry. Therefore, testing for drugs of abuse is paramount, and those analytical procedures employed are evolving in sophistication, specificity, and sensitivity.

Analytical methods to screen for drugs of abuse range in their sensitivity, specificity, and cost. By far, the most common analytical measure employed to screen for drugs of abuse is the immunoassay.

Analytical Methods

Immunassays
Of all the immunoassays, the enzyme multiplied immunoassay technique (EMIT) assay is the most frequently used due to its relatively rapid turnaround time and its low level of cost.1-3 Because of this, the EMIT assay also happens to be the screen of choice in pre-employment drug screening. The EMIT assay is a competition-based assay in which analyte in a patient's sample will compete for an antibody with analyte that is enzyme-bound. If the analyte-enzyme forms a complex with the antibody, the enzyme loses its activity. However, if the free analyte binds to the antibody, then the analyte-enzyme will be free to react with substrate, thus facilitating a biochemical reaction, thereby allowing indirect measurement of analyte.

There are, however, significant drawbacks to the EMIT assay. Certain food products and drugs can interfere with the test leading to false negatives and/or false positives. Furthermore, other drugs that structurally resemble the analyte of interest could generate a false positive result via cross-reactivity. Another major drawback of using the EMIT assay, as with all immunoassays, is that a number of additives can be placed in the sample to generate false negative results.

The radioimmunoassay (RIA) is similar to the EMIT assay but utilizes radioactive substrates for analysis.4-5 Because of the radioactive substrates, however, this methodology tends to be a less popular technique. There exist several drawbacks, such as the fact that one must possess a license to handle radioactive substances along with having to close off an area in the laboratory where radioactivity is used. Moreover, waste disposal of radioactive agents can be a cumbersome task due to strict disposal guidelines. Another shortcoming is that the radioactive reagents tend to have short half-lives, thus requiring quick use. Finally, when it comes to radioactivity, those performing the assays tend to express reluctance due to the fact of having to handle radioactive materials.

However, the quandary still exists regarding the interference and/or cross-reactivity of ingested foods/drugs, thereby contributing to false positive/negative results. Typically, an initial drug screen involves the utilization of an immunoassay due to its feasibility and low cost; unfortunately, it is this very assay that appears to be interfered with the most. False results from these initial screens can have serious implications towards a person's employment and leave a lasting stigma. This review attempts to highlight those analytical procedures used to screen for drugs of abuse along with listing those ingested foods/drugs that can potentially interfere with those analytical methods.

Thin Layer Chromatography (TLC)

Thin layer chromatography is a much older analytical technique that is not commonly used and requires considerable skill by the technician involved to analyze the results.6-7 The relative position of the spot can be characteristic of the specific substance while the diameter and intensity of the spot can be related to the amount of material present in the spot. Although capillary action establishes a distinctive separation pattern for each drug, this test will often produce subjective and quantitative results. Furthermore, chemical-masking agents may produce false positives, thus reducing accuracy and minimizing the chance of detection. Advantages include low cost, easy testing procedures, and that TLC can test for multiple drugs.
Gas Chromatography-Mass Spectrometry (GC-MS)

Above all, gas chromatography-mass spectrometry (GC-MS) is the analytical method with the highest degree of specificity for drug detection. This method is often used to confirm the results from other analytical methods. This method is a combination of 2 different analytical techniques: gas chromatography physically separates the various substances that have been extracted from a specimen (such as urine) while mass spectrometry is the technique used to provide a positive identification of substances that were separated by the gas chromatograph.

In general, GC-MS analysis involves using a solid phase or solvent-solvent extraction procedure to extract a drug and/or its metabolites from most other components of a urine specimen. After the extraction procedure, the extract is injected into the GC-MS to perform the final separation, identification, and quantification of the specific drug or drug metabolite present in the urine specimen. Due to the high degree of separation and elevated sensitivity of GC-MS, this method is highly sensitive for metabolites in low concentrations.

As with all analytical methods, there are limitations to using GC-MS. For example, the metabolite of interest must be volatile to facilitate transfer from the solid phase to the mobile carrier gas and thus elute from the analytical column to the detector. Size restrictions are also a factor in the applicability of GC-MS. Other drawbacks include high costs, longer analysis time, and the requirement of highly trained staff and sophisticated laboratory equipment. A summary of the main analytical methods is summarized in Table 1.

Traditionally, confirmational analysis of drugs of abuse is facilitated by GC-MS. However, this procedure can be both labor-intensive and time-consuming, as many drugs require considerable sample pre-treatment and derivatization before they can be analyzed by GC-MS. An advanced analytical technique that can circumvent these problems is liquid chromatography tandem mass spectrometry (LC-MS-MS). This technique is particularly useful for polar, non-volatile, thermally labile compounds that are difficult to analyze by GC-based techniques. With LC-MS-MS, for example, drugs of abuse such as amphetamines, opiates, benzodiazepines, GHB and many others can be analyzed without complex sample pre-treatment or derivatization.

Specimen Integrity

A single urine specimen is commonly screened for a wealth of drugs of abuse. A caveat, however, of using a urine specimen is that the collection process often serves as an opportunity for adulteration, substitution, and/or dilution. Therefore, strict measures have been implemented to ensure the careful collection of the urine specimen in order to maintain the specimen's integrity. Moreover, pre-analytical screening such as appearance, smell, temperature, measuring specific gravity, creatinine, and pH of the urine sample can determine as to whether substitution/adulteration has taken place. However, clinicians also need to be cognizant that adulteration of samples can also occur via the ingestion of adulterants prior to screening. Although there exists more and more sophisticated methodologies to screen for drugs of abuse, ingestion of common products can contribute to false negative and false positive results. The remainder of this review will attempt to highlight those commonly encountered products that can interfere or cross-react with the most frequently utilized methodologies employed to screen for drugs of abuse.

Foods Inducing Either Interference or Cross-Reactivity

The ingestion of those food products containing poppy seeds will affect both EMIT assays, TLC, and GC-MS analyses. The test results generated from a person who has consumed elevated levels of poppy seeds will indicate a false positive for opiates. This is due to the fact that poppy seeds contain trace amounts of morphine and codeine. However, criteria such as comparing the morphine-codeine ratio along with looking for other metabolites such as 6-acetylmorphine and acetylcodine can assess as to whether illicit use has taken place. A drink producing false positives, specifically for cocaine, is coca leaf tea. This product tends to contain trace levels for cocaine thereby contributing to a false positive using immunoassays and GC-MS. It is believed that the consumption of golden seal tea can also have an effect towards immunoassay-based drug screening because certain components in the tea can interfere with THC (tetrahydrocannabinol) detection contributing to false negative results; however, scientific studies have concluded that this is unfounded.

Drugs Contributing to Interference or Cross-Reactivity

Ibuprofen

Ibuprofen, a non-steroidal anti-inflammatory drug, is believed to interfere with a number of analytical methods. For example, it was shown that NSAIDs cause false positive results.

Table 1 The Comparison of the Major Analytical Methods Utilized in Screening for Drugs of Abuse

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoassays</td>
<td>Color/radioactivity/light change after reaction to drug metabolite</td>
</tr>
<tr>
<td>Thin Layer Chromatography</td>
<td>Capillary action establishes a distinctive separation pattern for each drug based on size, charge, etc.</td>
</tr>
<tr>
<td>Gas Chromatography-Mass Spectrometry</td>
<td>High degree of chemical separation and characterization of structures.</td>
</tr>
<tr>
<td>Other Chemicals</td>
<td>Other chemicals may produce &quot;false positives,&quot; low accuracy may produce &quot;false negatives,&quot; chemical masking agents reduce accuracy and minimize chance of detection.</td>
</tr>
<tr>
<td></td>
<td>High cost, takes longer for results, requires highly trained staff and sophisticated laboratory, should always be used to confirm any suspected &quot;positive&quot; test result using other technologies.</td>
</tr>
</tbody>
</table>
for cannabinoids using an EMIT assay and false positive results for both barbiturates and benzodiazepines by FPIA. It should be emphasized, however, that ibuprofen poses only a modest impact on drug analyses indicating that a small likelihood of a false positive immunoassay test of cannabinoids, benzodiazepines, or barbiturates exists after acute or chronic ingestion of NSAIDs. Moreover, NSAIDs have also been shown to cause false negative results for MS confirmation of cannabinoids.

Salicylates

Regarding other analgesics is the potential of salicylates to interfere with drug testing. It has been shown that the ingestion of aspirin can have a significant effect towards the detection of the cocaine metabolite benzoylecgonine, using the EMIT immunoassay, leading to false negative results. The investigators concluded from this study that this interference could be grounded on 2 possible premises: either metabolic products of acetylsalicylic acid produce a decreased signal in the urine or ingestion of the aspirin induces the excretion of other substances whose presence similarly affects the EMIT immunoassay. It was later shown by the same investigators that this interference was due to the primary urinary metabolite salicylic acid. This metabolite was shown to interfere with the measurement of NADH formed in the assay by reducing the molar absorptivity of NADH at 340 nm. Their studies concluded that measuring the EMIT assay signal at a wavelength at 376 nm eradicates the interference due to salicylic acid while maintaining the integrity and precision of the assay.

Dextromethorphan

Dextromethorphan, a common cough suppressant found in cough syrups has been implicated in causing false positives for the detection of phencyclidine (PCP). Furthermore, dextromethorphan has also been implicated in cross-reacting with immunoassays for opioids, presumably due to structural similarities.

Fluconazole

Wu and Dasgupta demonstrated that the antifungal medication fluconazole significantly interferes with GC-MS confirmation of cocaine. Fluconazole was shown to co-elute with benzoylecgonine, the chief metabolite of cocaine metabolism. It is also known that fluconazole will not interfere with the EMIT assays. Dasgupta and colleagues found that this co-elution plight could be curtailed via chemical modification of benzoylecgonine to the corresponding pentafluoropropionyl derivative. The interference of fluconazole was shown to be completely eliminated; the pentafluoropropionyl derivative of benzoylecgonine eluted at 14.7 minutes, whereas the derivatized fluconazole eluted at 15.6 minutes. Moreover, the mass spectral fragmentation pattern of derivatized benzoylecgonine was particularly unique from the mass spectral features of derivatized fluconazole, in both electron ionization and chemical ionization mode of operation of mass spectrometers.

Ephedrine

The herbal plant ephedra (Mahuang) along with any ephedra-containing products were removed from shelves due to a mandated policy by the FDA. This herb contains ephedrine, which has been associated with a multiplicity of detrimental cardiovascular effects and was even implicated in a number of deaths. Ephedrine and pseudoephedrine (found in many over-the-counter cold medications) have been shown to cross-react with initial immunoassays, thereby producing false positive results for amphetamine and methamphetamine due to their structural similarities. Subsequent GC-MS analyses, however, can make the differentiation.

Tolmetin

Tolmetin, a NSAID, is used to reduce the pain, inflammation, and stiffness caused by rheumatoid arthritis and osteoarthritis. This medication, however, has also demonstrated significant interferences in the EMIT assay in detecting opiates, cannabinoids, and amphetamines. It was shown that urine samples containing elevated concentrations of tolmetin had characteristic high molar absorptivity at the wavelength utilized in EMIT assays (340 nm), thus resulting in error alarms on the instrumentation. Samples containing opiates and cannabinoids tested negative, while instrument error alarms were seen with those urine samples containing amphetamines. The presence of tolmetin in urine samples, however, did not affect GC-MS interpretation. It was therefore inferred that the presence of tolmetin in urine samples could lead to the interference of the EMIT assay for drugs of abuse leading to false results.

Narcotic Analgesics

There are a number of legal narcotic drugs that will cross-react with immunoassays leading to the generation of false positive results for opiates. Codeine, hydrocodone bitartrate (cough syrups), and oxycodone HCl are the most common narcotics using the initial immunoassay screens due to their structural similarities. Subsequent GC-MS analyses can make the discrepancy between these medications and illicit drugs. Although these drugs are legal, it is more and more common to find those individuals who are abusing these drugs due to their narcotic properties, which engenders potential problems in discriminating those individuals who are taking these medications for therapeutic purposes versus recreational use.

Fenfluramine/Phentermine

Fenfluramine and phentermine are weight loss medications that can also produce false positive results for amphetamines using the initial immunoassay screens due to cross-reactivity, but again can be differentiated using GC-MS. Like the narcotic analgesics, structural similarities between fenfluramine/phentermine and the amphetamines contributes to this cross-reacting.

Conclusion

There are a number of medications can that interfere and cross-react with analytical screens in the detection for drugs of abuse (Table 2). It is important to emphasize that the aforementioned list of substances is not comprehensive; there are a number of suspected interfering and cross-reacting substances, but due to a paucity of sufficient empirical data, the probability of there being interference or cross-reactivity is not deemed significant.

Typically, those foods or drugs interfering with drug screening will affect immunoassays due to interference or cross-reactivity. The utilization of GC-MS and the emergence of more sophisticated techniques such as LC-MS-MS,
however, are often used as confirmatory methods to eliminate any possibilities of false negative/positive results. It is therefore incumbent on the clinician to assess the full medical/historical data of the patient along with any dietary factors that could possibly hinder the results of a screen for drugs of abuse.


### Table 2: A Summary of Those Ingested Substances That Can Interfere With Drug Screens

<table>
<thead>
<tr>
<th>Interfering Agent</th>
<th>Result</th>
<th>Basis for Interference or Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poppy seeds</td>
<td>False positive for opiates using immunoassays, TLC, and GC-MS</td>
<td>Contains trace amounts of morphine and codeine</td>
</tr>
<tr>
<td>Coco leaf tea</td>
<td>False positive for cocaine using immunoassays and GC-MS</td>
<td>Contains trace amounts of cocaine</td>
</tr>
<tr>
<td>Golden seal tea</td>
<td>False negative for THC using immunoassays</td>
<td>Mechanism still unclear; possibly due to interference</td>
</tr>
<tr>
<td>Mahuang/Ephedrine</td>
<td>False positive for amphetamines using immunoassays</td>
<td>Structural similarities will result in cross-reactivity</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>False positive for barbiturates, benzodiazepines, and cannabinoids using immunoassays</td>
<td>Believed to cause interference with the immunoassays</td>
</tr>
<tr>
<td>Salicylates</td>
<td>False negative for cocaine using immunoassays</td>
<td>Metabolite of acetylsalicylic acid causes interference with measurement</td>
</tr>
<tr>
<td>Dextromethorphan</td>
<td>False positives for PCP and opioids using immunoassays</td>
<td>Mechanism still unclear; structural similarities possibly causing cross-reactivity</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>False negative for opiates, amphetamines, and cannabinoids using immunoassays</td>
<td>Fluconazole co-elutes with benzoylglucine (main cocaine metabolite)</td>
</tr>
<tr>
<td>Tolmetin</td>
<td>False positive for barbiturates, benzodiazepines, and cannabinoids using immunoassays</td>
<td>Similar molar absorptivity interferes with drugs of abuse detection</td>
</tr>
<tr>
<td>Narcotic analgesics (ie, hydrocone, xraydcone)</td>
<td>False positive for opiates using immunoassays</td>
<td>Structural similarities will result in cross-reactivity</td>
</tr>
<tr>
<td>Fenfluramine/Phentermine</td>
<td>False positive for amphetamines using immunoassays</td>
<td>Structural similarities will result in cross-reactivity</td>
</tr>
<tr>
<td>Dextromethorphan</td>
<td>False positive for opiates using immunoassays</td>
<td>Contains trace amounts of morphine and codeine</td>
</tr>
<tr>
<td>Phentermine</td>
<td>False positive for amphetamines using immunoassays</td>
<td>Contains trace amounts of cocaine</td>
</tr>
<tr>
<td>Meperidine</td>
<td>False positive for amphetamines using immunoassays</td>
<td>Structural similarities will result in cross-reactivity</td>
</tr>
<tr>
<td>Methadone</td>
<td>False positive for benzodiazepines using immunoassays</td>
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**Note:** This table provides a summary of ingested substances that can interfere with drug screens. It is important to note that the list is not exhaustive and that additional substances and conditions may also cause false results.