

## Methods Based on Thin Layer Chromatography (TLC) Usage to Identity Counterfeit Drugs

Dr. Gary Witman  
Dr. Mark Moskovitz

Identification of counterfeit drugs has become a serious international issue. By definition counterfeit drugs are mislabeled medicines with substandard safety, quality and effectiveness. Counterfeit products include drugs with correct or wrong ingredients, without active ingredients, with insufficient active ingredients or fake packaging.

The counterfeiting of pharmaceuticals has only been detected for the past 20 years. The poor quality of drugs has been linked to counterfeiting of medicines, chemical instability (especially in tropical climates) and poor quality control during manufacture. Pharmaceutical compounds have specific biological properties and mechanisms of action and individuals are dependent upon consistent and reproducible drug performance. In most countries the quality of pharmaceutical products is assured through inspection systems by governmental regulatory agencies and by the utilization of current good manufacturing (cGMP) practices. Counterfeit drugs may be placebo in quality, or worse containing materials that do harm.

The World Health Organization estimates that 25% of medicines in developing countries are counterfeit, with a rate as high as 50% in some places. During 2006 the European Commission reported that customs agents intercepted 1.5 million counterfeit drugs at EU borders – an increase of 384% from a year earlier. In just two months in 2008 European Union officials seized more than 34 million fake prescription pills. The problem with fake and suboptimal medications has been much worse with the advent of the World Wide Web, as a significant proportion of medicines sold through Internet pharmacies are counterfeit. The most common counterfeit drug agents are those that are prescribed for the treatment of malaria, tuberculosis, HIV, and other opportunistic infections. Furthermore, it has been stated that the greatest health risk in the world today is the lack of appropriate medicines for treating both common and life threatening illnesses. It is known that some counterfeit drugs have caused kidney failure in patients. The growing challenge is halting the distribution and marketing of such medicines at every level of the distribution chain.

To understand the magnitude of the problem, the US Center for Medicine in the Public Interest has predicted that this year, the global sales of counterfeit drugs will reach \$75 billion USD, which is a 95% increase in just the past few years. At least 20% of the Indian drug supply is fake. Concurrently the World Health Organization believes that India is responsible for at least 35% of the world's counterfeit medications. A recent study in the British medical journal The Lancet, reported that around 40% of the drug artesunate, used for the treatment of malaria, was a complete inert placebo in pills being sold in Thailand and Nigeria. In Southeast Asia and Africa the preferred counterfeit antimicrobials appear to be older antibiotics – such as penicillin, tetracycline, trimethoprim-sulfamethoxazole, quinolones, chloramphenicol and anti-malarials such as chloroquine, mefloquine, quinine and artesunate, that are globally no longer patent protected.

Almost everything connected with drug manufacturing is being counterfeited – the active ingredients, dosage form, package inserts, packaging, manufacturer's names, batch numbers, expiration dates and documentation relating to quality control. Fake pharmaceuticals often have the same appearance as the brand name and generic drugs they mimic. They are often indistinguishable in packaging, pill color, shape and markings. Indeed, they have electronic codes, that pharmacists scan to verify drug authenticity.

### Main Types of Counterfeit Drugs

| Categories of Drugs | % of Total Counterfeits |
|---------------------|-------------------------|
|---------------------|-------------------------|

## Lead Removal from Water by the U

|                                 |    |
|---------------------------------|----|
| Antibiotics                     | 28 |
| Hormones and Steroids           | 18 |
| Anti-asthma and anti-allergy    | 8  |
| Anti-malarial                   | 7  |
| Analgesics and anti-pyretics    | 6  |
| Others (14 therapeutic classes) | 33 |

The Chinese are scared of the safety of their domestic drug supply, and with good reason. As many as 5 million Chinese work in the drug counterfeit market, that includes fake drugs, patent infringement and trademark infringement. In January, 2009 two people died and nine were hospitalized using counterfeit diabetes drugs in Xinjiang province. Fake products are often deliberately engineered to pass basic quality tests, using a substitute ingredient that mimics the chemical activity of the authentic ingredient closely. In 2008 a chemical added to heparin as a blood thinning agent was responsible for at least 95 deaths in China, Europe and the United States. In March 2009 the United Kingdom seized nearly \$750,000 of counterfeit pharmaceuticals which originated in China.

To counter this enormous commitment to illegal drugs, the Chinese government has spent nearly \$ 1 billion through their State Food and Drug Administration to monitor illegal drug production and has shut down more than 1,000 illegal drug facilities. Despite these efforts, China's intellectual property laws are not yet consistent or compliant with the World Trade Organization's international Intellectual Property agreement. China still allows drugs that infringe trademarks and may be substandard to be resold (after repackaging) rather than being destroyed. China implemented a Fast Chemical Identification System in 2005, which is usually targeted at the molecular structure functional groups in the drug molecule. This is comprised of two color reactions based on functional groups in the molecules and two TLC methods for screening of fake macrolide antibiotics.

India has taken a step forward by agreeing to partner with the Food and Drug Administration (US) to allow more foreign inspectors. And the FDA has now placed eight full time permanent positions in China.

Thin layer chromatography is the main screening method being used today in order to determine if a drug product meets label specifications and is legal. TLC can be employed for the identification of drug substances, to estimate substance content and to detect related substances that may be regarded as impurities. This technique allows the active ingredient to be recognized by comparison with a known drug standard. The approach using TLC chromatography is cheap, specific and sensitive. The TLC procedures are therefore preferred, as they are capable of giving semi-quantitative information on the active ingredient and also on any related substances in the dosage form.

### **The Center for Disease Control (CDC, Atlanta, GA) recommends testing for suspicious drugs, especially for travelers.**

According to the CDC, "Drug quality can be evaluated in the field by two simple, effective and low cost techniques: thin layer chromatography and colorimetry...the TLC technique consists of placing a spot of drug sample on a thin layer of silica attached to a plate of glass, aluminum or plastic. The plate is then inserted into a vessel containing solvent mixture. By capillary action, the solvent creeps up the adsorbent material, silica, alumina, or cellulose, and dissolves the sample. The drug sample consists of a mixture of drug and inactive ingredients. These compounds have various affinities to the adsorbent matrix and will migrate with the solvent at various speeds. This character effectively separates out a mixture of compounds. After migration of the solvent is complete, individual components can be visualized by chemical treatment or ultraviolet (UV) absorbance. The distance that the components migrate is characteristic for each compound; therefore the active ingredient can be recognized by comparison with a known drug standard. The solvent can be modified to increase resolution between various components. This method is relatively inexpensive, specific and sensitive. It is commonly used to assess drug quality."

## Lead Removal from Water by the U

Over 300 German Pharma Health Fund e.V. Mini-labs (GPHF-Minilab) are being used in 70 countries to help identify counterfeit and substandard drugs. These minilabs, which contain simple materials (as described below) and that utilize standard TLC technology are able to analyze some 40 commonly adulterated counterfeit drug compounds. One thing unique about the GPHF minilab is that iodine-KI solution is replaced by iodine vapor sublimed from crystals. Minilab supplies a collection of authentic secondary standard tablets and capsules in sealed plastic tubes. The Mini analysis identifies the active ingredient by comparison of distance of travel (RF value) between the sample spot and an authentic standard spotted on the same plate, and semi-quantitative proof of content is made visually comparing the color, size, and intensity between the sample spot and reference spots for each method of detection.

In response to effective anti-counterfeit measures, counterfeiters have often introduced small quantities of the genuine pharmaceutically active substances into the dosage forms. TLC procedures are able to provide semi-quantitative information on the active ingredient and also on the related substances, providing valuable information on the drug being tested. When the counterfeit compound is extremely sophisticated in design, it may require more advanced analytical tools such as elemental analyzer-isotope mass spectrometry (EA-IRMA), LC/MS, Raman spectrometry, X-ray powder diffraction method, near infrared spectrophotometry (NIR) and nuclear magnetic resonance to help more carefully define the drug contents. NIR has become portable, but unlike TLC, this technique is not quantitative. However, the use of these more sophisticated technologies is beyond the scope of this paper.

Separations to determine the activity of drug agents can be performed on for example silica gel layers containing a fluorescent indicator, and separated spots are detected under ultraviolet lamps or with iodine detection reagent. This technology can be performed out in the field by inspectors with a limited scientific expertise. Development and identification detection are carried out either in glass jars or in polyethylene bags. Tablets are ground into a fine powder and a proper volume of solvent is added.

In most instances TLC identification is carried out on multi channeled soft layer silica gel GF254 plates or hard layer silica gel F254 plates (20 cm x 20 cm). Mobile phases may consist of a multitude of solvent systems depending on the class of drugs such as hexane, ethyl acetate, toluene, alcohols, ammonia, and others. TLC plates are allowed to dry. If a more elaborate TLC separation is required the plate can be run in a two dimension solvent system.

The presence of a fluorescent indicator is necessary for the detection of drugs that quench fluorescence under 254 nm UV light. Blue fluorescent quenched spots may be viewed on a bright green background under 254 nm ultraviolet (UV) light and of brown spots in white light after dipping plates in iodine-KI solution or exposing the plates to an iodine vapor mist. The staining of a Thin Layer Plate with iodine vapor is among the oldest methods for the visualization of organic compounds. It is based upon the observation that a iodine vapor has a high affinity for both unsaturated and aromatic compounds.

Dynamic Adsorbents provides the widest range of TLC plates in the industry and can assist you with your use of this technology.

**[Back to HTML article](#)**