Gazi University Journal of Science
GU J Sci
26(3):407-417 (2013)

Spectroscopic Analytical Methods for Detection of Counterfeit Pharmaceutical Preparations – A Mini-Review

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Received: 15.08.2102 Revised: 04.10.2012 Accepted:08.10.2012

ABSTRACT

Pharmaceutical counterfeiting is becoming a serious problem all over the world. This paper presents several of the most important methods used to elucidate this problem including near- and mid-infrared spectroscopy, Raman spectroscopy, isotopic characterization, chromatographic and mass spectrometric approaches.

Key Words: counterfeit drugs; spectroscopic methods ; pharmaceutical drugs; herbal medicines.

1. INTRODUCTION

The counterfeiting of medicines has been known of since around 1990 and the problem has escalated - in both developing and developed countries. Many more cases are appearing, not only in the developing world but, increasingly, in developed countries. The increased occurrence of counterfeit medicines has several serious consequences. These may include illness or death of patients, higher medical insurance and lost revenues to pharmaceutical manufacturers and governments.

The World Health Organization (WHO), estimated that 5–7% of pharmaceutical products worldwide are counterfeit goods, has defined counterfeit drugs as those which are ‘‘deliberately mislabeled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products with counterfeit products including drugs with the correct ingredients or with the wrong ingredients; without active ingredients, with insufficient active ingredient or with fake packaging’’1.The true and false identification could be performed with specific peaks and their absorption

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ratios. Adulteration is a global phenomenon affecting a wide range of products, spreading at an alarming rate to electrical equipment, cigarettes, and even medicines2.

The objectives of this article are to present some of the most relevant papers related to analytical methods used in adulterated products analysis covering the period between 2005 and 2011. Prior to a review on this subject, it is useful to give a short introduction to the concept of adulteration in pharmaceutical products. In the major section qualitative determinations of adulterated products in pharmaceutical and herbal products will be presented including technques using near- and mid-infrared, Raman spectroscopy among others.

2. COUNTERFEİT CONCEPT

A recent report from the International Pharmaceutical Students' Federation3 has indicated that some drugs are more counterfeited than others. High-consumption, expensive and innovative drugs along with well-established generic drugs are most readily affected. The main categories include antibiotics, hormones and steroids, although any therapeutic class may be copied. Furthermore, it is known that almost everything connected with the drug manufacture process is being counterfeited viz. active ingredients, dosage forms, package inserts, packaging, manufacturers' names, batch numbers, expiry dates and documentation relatwed to quality control 4.

Counterfeit products are often produced with the intent to take advantage of the superior value of the imitated product. The word counterfeit frequently describes both the forgeries of currency and documents, as well as the imitations of works of art, toys, clothing, software, pharmaceuticals, watches, electronics and company logos and brands. In the case of goods, it results in patent or trademark infringement. Additionally, it is fairly common in big cities for would-be criminals to sell counterfeit illegal drugs, such as a bag of pure baking soda sold as cocaine or heroin, or a bag of oregano sold as marijuana. This takes advantage of the extremely high prices of illicit drugs and the relatively low prices of common materials such as baking soda and oregano, as well as taking advantage of the similarity in appearances that certain house-hold items share with certain illicit drugs.

The increase in the number of counterfeits penetrating into the open market has created the need for a product authentication approach in tracing and tracking the product anytime, anywhere.

For the purpose of this review, any pharmaceutical product will be considered to be adulterated if:

1. any valuable constituent has been in whole or in part omitted;
2. any substances has been substituted wholly or in part therefore;
3. damage or inferiority has been concealed in any manner, or
4. any substances has been added thereto or mixed therewith so as to increase its bulk or weight or reduce its quality or strength, or make it appear better or of greater value than it is5,6.

The quality of a determination of authenticity is clearly linked to number and types of tests performed. Each time a useful new method has been introduced, previously undetected adulterations have surfaced. Unfortunately, the new methods generally do not supersede older methods and represent additional authentication costs.

The problem of counterfeit drugs is important all over the world. For the first time the World Health Organization (WHO) obtained information about forgeries in 1982. At that time counterfeit drugs were mainly found in the developing countries. The definition for "counterfeit drug" by WHO is as follows: "A counterfeit medicine is one which is deliberately and fraudulently mislabeled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit products may include products with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredient or with fake packaging"7.

Nowadays, there are "high quality" counterfeit drugs that are very difficult to detect. It is worth mentioning that fake drugs include dietary supplements too.

Since pharmaceutical preparations counterfeiting is primarily a form of visual deception, the researchers in the adulteration field tend to focus most of their energies on replicating the things that can be easily seen and checked. Therefore, packaging quality is usually high (and fake packaging is often very hard to distinguish from that of the genuine product) but the dosage form itself is often substandard. Even if it has some active ingredient, the product is unlikely to have the same physical or chemical profile as the genuine formulation. One of the most powerful and direct methods to detect adulteration products, therefore, is to analyze the properties of the product itself. This can either be achieved using laboratory-based testing of purchased or seized samples, or can increasingly be done using portable, non-invasive techniques. These analytical methods can also be applied to packaging, but are especially effective when used to validate the active ingredients and chemical constitution of the dosage form itself. Because these techniques are based only on physical or chemical properties of the product, they are extremely difficult for criminals to circumvent using counterfeit materials. Although not all of the techniques used in the factory QA process are suited to the routine search for counterfeits, there are many which can be adapted successfully. The potential methods that are available ranging from simple and cheap in-field ones (colorimetry and thin layer chromatography) to more advanced laboratory methods (mass spectrometry, nuclear magnetic resonance, and vibrational spectroscopies) through chromatographic methods, which remain the most widely used.

Express-methods for detection of counterfeit drugs are of vital necessity. Visual control, dissociating tests or simple color reaction tests reveal only very rough forgeries.

The Global Pharma Health Fund (GPHF) is a charitable organization initiated and funded exclusively by donations from Merck, Darmstadt · Germany. Within international development assistance, the GPHF aims to improve health care, the work currently supporting the fight against counterfeit medicines proliferation using the GPHF-Minilab®. The GPHF website8 provides more details of the tests and the drugs covered. A four-stage process is used to test for the quality of drugs:

(a) visual inspection of solid dosage forms and packaging material;

(b) tablet and capsule-disintegration test for a preliminary assessment of any deficiencies related to drug solubility;

(c) simple color reactions to identify drugs;

(d) semi-quantitative TLC to check for quantities of drug present.

The growth of pharmaceutical counterfeiting is a major public health problem. This growth is resulting in a proportional increase in the number of samples that medicines control laboratories have to test. Thus the need for simple and affordable preliminary screening methods to be used by inspectors to decide in the field whether to collect a sample for further laboratory analysis or not.

The battle against counterfeit drugs has only just begun and it will be a long road ahead for those involved in getting rid of this illegal trade. Peter Lowe of the ICC's Counterfeiting Intelligence Bureau has concluded that: ''Despite existing regulatory and legal efforts, the counterfeiting of pharmaceuticals remains a very serious public health concern.''10

3. PHARMACEUTİCALCOUNTERFEİT DRUGS

Several methods are employed to detect drugs which may be suspect while researchers are working assiduously to develop other rapid detection schemes. They range from simple thin layer chromatography (TLC) to more sophisticated techniques such as Fourier transform infrared spectroscopy (FTIR) and liquid chromatography-mass spectrometric (LC-MS) approaches.

The literature studied shows a great number of papers dedicated to pharmaceutical counterfeits drug analysis using different analytical techniques. There are some reviews dedicated to this subject11-16. Some of them were dedicated to several analytical techniques such as: general methodes11,12, Fourier Transform Infrared Spectroscopy13,14, X-ray spectroscopy15, Raman spectroscopy16, NIR spectroscopy17 and respectively chromatographic methods4 or are related to some specific products such us melamine adulteration scandal19 and respectively heparin contaminants20.

Next some qualitative determinations of active principle ingredient (API) content in different adulterated pharmaceutical preparations dosage forms will be presented in alphabetic order of the API.

Antibiotics, among the most widespread drugs, have been particularly targeted by counterfeiters. A fast chemical identification system consisting of two color reactions based on functional groups in molecules of macrolide antibiotics and two TLC methods was developed for screening of fake macrolide drugs21. Sulfuric acid reaction as a common reaction of macrolides was first used to distinguish the macrolides from other types of drugs and then 16-membered macrolides and 14-membered ones were distinguished by potassium permanganate reactions depending on the time of loss of colour in the test solution; after which a TLC method carried out on a GF254 plate (5 cm×10 cm) was chosen to further identification of the macrolides. A suspected counterfeit macrolide preparation can be identified within 40 min. The system can be used under different conditions and has the virtues of robustness, simplicity and speed. Gaudianoet al22 presents a single method for the simultaneous analysis of some of the most common and counterfeited essential antibiotics: ampicillin, amoxicillin + clavulanic acid, doxycycline, cloxacillin, chloramphenicol. A full validation was performed in terms of linearity, precision, robustness and trueness; an assessment of uncertainty was carried out exploiting these data. A wide linearity range was investigated considering the specific nature of counterfeit and sub-standard drugs, whose content in active substance may be rather far from the declared amount. A large span in robustness parameters was considered and a complete intermediate precision assessment was conducted, envisaging the possibility of transferring the method to quality control laboratories, hopefully in developing countries. Finally, the method was successfully applied to the analysis of antibiotics purchased on the informal market in Chad, among which counterfeit and sub-standard samples were detected. The aim of the study presented by Sanliet al23 was to develop a fast, sensitive and reliable method for rapid screening of cephalosporin injectable dosage forms, namely ceftazidime and ceftizoxime, to the detection of counterfeit and substandard drugs that might be illegally commercialized. Aqueous pKa values of studied compounds have been determined by UV-spectrophotometry and liquid chromatography were used for the determination and direct characterization of the dissociation constants by using the dependence of the capacity factor on the pH of the mobile phase in 20% (v/v) methanol–water binary mixture in which separation was performed. The method was shown to be linear, sensible, accurate, and reproducible over the range of analysis and it can be used to pharmaceutical formulations containing a single active ingredient within a short analysis time.

Malaria continues to be a major cause of mortality in third world countries with 1–2 million deaths each year. In support of the efforts to combat the illegal sale and distribution of counterfeit anti-malarial drugs, Ricci et

al24 evaluated a new analytical approach for the characterization and fast screening of fake and genuine artesunate tablets using a combination of Raman spectroscopy, Spatially Offset Raman Spectroscopy (SORS) and Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) imaging. The methodology performance was evaluated in terms of specificity, sensitivity and precision. Vibrational spectroscopy provided chemically specific information on the composition of the tablets; the complementary nature of Raman scattering and FTIR imaging allowed the characterization of both the overall and surface composition of the tablets. The depth-resolving power of the SORS approach provided chemically specific information on the overall composition of the tablets, non-invasively, through a variety of packaging types. Spatial imaging of the tablet surface (using ATR-FTIR) identified the location of domains of excipients and active ingredients with high sensitivity and enhanced spatial resolution. The advantages provided by a combination of SORS and ATR-FTIR imaging in this context confirm its potential for inclusion in the analytical protocol for forensic investigation of counterfeit medicines. In another paper Ricci et al25 used a combination of Fourier-transform infrared (FTIR) spectroscopic imaging and desorption electrospray ionization linear ion-trap mass spectrometry (DESI MS) for characterization of counterfeit pharmaceutical tablets. The results obtained revealed the ability of FTIR imaging in non-destructive micro-attenuated total reflection (ATR) mode to detect the distribution of all components in the tablet, the identities of which were confirmed by DESI MS. Combination of these two orthogonal surface-characterization methods has great potential for detection and analysis of counterfeit tablets in the open air and without sample preparation. Green et al26 have evaluated refractometry, colorimetry and a technique combining both processes as simple and accurate field assays to rapidly test the quality of the commonly available antimalarial drugs; artesunate, chloroquine, quinine, and sulfadoxine. Method bias, sensitivity, specificity and accuracy relative to high-performance liquid chromatographic (HPLC) analysis of drugs collected in the Lao PDR were assessed for each technique. The HPLC method for each drug was evaluated in terms of assay variability and accuracy. The accuracy of the combined method ranged from 0.96 to 1.00 for artesunate tablets, chloroquineinjectables, quinine capsules, and sulfadoxine tablets while the accuracy was 0.78 for enterically coated chloroquine tablets. These techniques provide a generally accurate, yet simple and affordable means to assess drug quality in resource-poor settings. Near-infrared spectroscopy (NIRS) is a powerful tool in pharmaceutical forensics, and it was tested for discriminating between counterfeit and genuine artesunate antimalarial tablets27. Using NIRS, it was founded that artesunate tablets could be identified as genuine or counterfeit with high accuracy. Multivariate classification models indicated that this discriminatory ability was based, at least partly, on the presence or absence of spectral signatures related to artesunate.

A simple high-performance liquid chromatography (HPLC) method with both ultraviolet (UV) and electrospray ionisation mass spectrometry (ESI-MS) detection has been developed for the determination of seven pharmaceuticals in counterfeit homeopathic preparations28. Naproxen, Ketoprofen, Ibuprofen, Diclofenac, Piroxicam, Nimesulide and Paracetamol were so separated by reversed phase chromatography and determinated. Linearity was studied with UV detection in the 50–400 μgmL−1 range and with ESI-MS in the 0.1–50 μgmL−1 range. Good correlation coefficients were found in both UV and ESI-MS. Detection limits ranged from 0.18 to 41.5 ng in UV and from 0.035 to 1.00 ng in ESI-MS. This method was successfully applied to the analysis of homeopathic preparations like mother tinctures, solutions, tablets, granules, creams, and suppositories.

A relatively new drug for which counterfeits have been traced is the cholesterol-lowering medicine Lipitor®. Authentic Lipitor® tablets contain atorvastatine as the active pharmaceutical ingredient (API) in a well-defined matrix29. Research has been carried on the feasibility of near infrared spectroscopy (NIRS) and Raman spectroscopy as rapid screening methods to discriminate between genuine and counterfeits of the cholesterol-lowering medicine Lipitor®. The discriminative power of the NIR model, in particular, largely relies on the spectral differences of the tablet matrix. This is due to the relative large sample volume that is probed with NIR and the strong spectroscopic activity of the excipients. PLS-DA models based on NIR or Raman spectra can also be applied to distinguish between atorvastatine and lovastatine as the API used in the counterfeits tested in this study. A disadvantage of Raman microscopy for this type of analysis is that it is primarily a surface technique. As a consequence spectra of the coating and the tablet core might differ. However, the robustness of the PLS-DA models turned out to be sufficiently large to allow a reliable discrimination. Principal component analysis (PCA) of the spectra revealed that the conditions, at which tablets have been stored, affect the NIR data. This effect is attributed to the adsorption of water from the atmosphere after unpacking from the blister. It implies that storage conditions should be taken into account when the NIR technique is used for discriminating purposes. Both models based on NIR spectra and Raman data enabled reliable discrimination between genuine and counterfeited Lipitor® tablets, regardless of their storage conditions.

Since the introduction of Viagra® for erectile dysfunction by Pfizer in 199830, numerous websites have been appearing, were people easily and anonymously can purchase Viagra® tablets. Viagra® has been falsified numerous times. So far, different methods have been used for the analysis of sildenafil in Viagra® tablets. These methods include: chromatography (LC-DAD-MS31, LC-ECI-MS/MS32 and LC-MS/MS33), X-ray powder diffraction34, Raman spectroscopy35-37, and respectively Fourier Transform Infrared spectroscopy (FTIR)37. The X-ray diffraction method34 turns out to be very fast and

reliable for detecting Counterfeit and imitation, and it correctly predicts the presence or absence of active substance and/or particular excipients. Raman35 spectroscopy is proposed as a fast and reliable method for the detection of counterfeit Viagra® tablets. This technique can easily differentiate genuine from counterfeit tablets without the need of sample preparation. Europe and North America are more and more confronted with the counterfeiting problem. In their study, Sacre et all35 used 26 counterfeits and imitations of Viagra® tablets and 8 genuine tablets of Viagra® which were analysed by Raman microspectroscopy imaging. After unfolding the data, three maps are combined per sample and a first PCA is realised on these data. PCA was applied as exploratory analysis tool on different spectral ranges to detect counterfeit medicines based on the full spectra (200–1800 cm−1), the presence of lactose (830–880 cm−1) and the spatial distribution of sildenafil (1200–1290 cm−1) inside the tablet. A good discrimination of genuine samples was obtained with multivariate analysis of the full spectra region (200–1800 cm−1). Comparing different techniques of vibrational spectroscopy (FTIR, NIR and Raman)37, only the regions between 1800-400cm−1 and 7000-4000cm−1 were used for FT-IR and NIR spectroscopy respectively. Partial Least Square analysis has been used to allow the detection of counterfeit and imitation tablets. It is shown that for the Viagra® samples, the best results were provided by a combination of FT-IR and NIR spectroscopy. On the other hand, the best results for tadalafil (Cialis®) samples were provided by the combination of NIR and Raman spectroscopy (1400-1190cm−1). These techniques permitted a clear discrimination between genuine and counterfeit or imitation samples but also the distinction of clusters among illegal samples. This might be interesting for forensic investigations by authorities. Some of these methods can also be used to monitor the exact content of oseltamivir in Tamiflu® capsules32.

Not only the active principles were counterfeited but also adulteration of pharmaceutical packaging containers with post consumer recycled plastic materials was considerably difficult to identify due to the similar chemical compositions of virgin and recycled plastics. Near-infrared (NIRS) spectroscopy coupled with conformity test was proposed to screen the adulteration of pharmaceutical packaging containers38. Also shopping bags, commonly encountered in the packaging of drug doses, were characterized by thickness measurements, infrared spectroscopy and differential scanning calorimetry39.

4. HERBAL COUNTERFEİT DRUGS

Herbal Medicine contains multiple botanicals, each of which has many compounds that may be relevant to the medicine's putative activity. Therefore, analytical techniques that look at a suite of compounds, including their respective ratios, provide a more rational approach to the authentication and quality assessment of these types of products.

In recent years, dietary supplements and herbal medicines are increasing in popularity all over the world. However, it is problematic that some manufacturers illegally included synthetic drugs in their products40. Due to the extremely complex matrices of those products, most existing methods for screening illegal adulterations are time-consuming and liable to false positive.

A significant increase in the use of herbal medicines and their preparations were observed all over the world. Adulterations with synthetic drugs are common problems with phytopharmaceutical products and this can potentially cause adverse effects. In consequence, it is important to determine the presence of synthetic drugs in herbal medicines to ensure their efficacy and safety. The efficiencies of herbal medicines depend on the amount of active components in them, which could vary significantly in contents. Therefore, the quality control of herbal medicines is a very important issue. There are many papers that summarizes the true and false identification, producing region identification and quality evaluation of herbal medicines.

Determining adulteration in food is accomplished by comparing analytical data with historical or control data. Without a proper reference set, the determination of adulteration is impossible. Adulteration is best shown by detecting a foreign component that is characteristic of a specific adulterant. Sometimes it is necessary to use deviations in a parameter, but typically these approaches are less sensitive owing to the natural variation in food. The decision not to perform a test that yields additional information increases the potential of erroneously accepting a sample.This proof of the negative challenge has led to competition between the practitioners of authentication and those who would adulterate their products or ingredients. This competition is readily apparent. Each time a useful new method has been introduced, previously undetected adulterations have surfaced.

The true and false identification could be performed with specific peaks and their absorption ratios. One of the most important method for discriminating the herbal drugs is the chemical fingerprint analysis. In this way there are many papers related to this subject, where different techniques were used. So we can mentione some of these techniques: infrared spectroscopy (FTIR)41-45, liquid chromatography46-51, gas-chromatography52, 53 and other related techniques54-60. Next we will present some of the most important papers related to herbal counterfeit drugs.

Ginseng, is a well known Chinese herb, that exists as two major varieties – Panaxquinquefolius L. (American) and Panax ginseng C.A. Meyer (Asian)61. It is considered adaptogenic to improve physical and mental performance. Ginseng is an expensive herb, and adulteration with other cheaper products may occur. Ginseng is a widely used medicinal product that grows mainly in Korea, China and America. American ginseng is classified as an endangered species, and so the import and export of this type of ginseng is illegal in certain

countries. Due to this restriction it is becoming increasingly important to be able to distinguish between different types of ginseng. Quality assurance of ginseng is needed since many of its commercial products now come in various formulations such as capsules, powder, softgels and tea. There is a report62 for a rapid means of distinguishing American and Asian ginsengs from two morphological fakes – sawdust and Platycodongrandiflorum, via pattern differences and principal component analysis of their infrared spectra. The results show that ginseng can be distinguished from both sawdust and Platycodongrandiflorum, hence there is a potential of using infrared spectroscopy as a novel analytical technique in the authentication of ginseng. FT-Raman spectroscopy63 has the ability to discriminate between ginseng specimens according to the country of origin and the effects of processing on the ginseng material. The ginsenoside content of ginseng differs in both conformation and concentration depending on the source of the ginseng, which means that ginseng grown in different countries should express unique spectral features. The presence or absence of these features, therefore, could indicate the geographical origin of the sample. Several spectral features were identified for a range of ginsengs, such as a peak at 980 cm−1 that was only found in Chinese ginseng, and the different wavenumber positions of characteristic ginseng bands near 1600 cm−1. This indicates that Raman spectroscopy can be used to pinpoint the origin of an unknown ginseng sample and that it would provide a rapid nondestructive analytical technique for formally discriminating between restricted and permitted imports. A new method using ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF-MS)64 was developed for the rapid qualitative and quantitative analyses of Asian ginseng (Panax ginseng C.A.Meyer) in adulterated American ginseng (Panaxquinquefolium L.) preparations within 2 min. The method was based on the baseline chromatographic separation of isomeric compounds of ginsenosideRf and 24(R)-pseudoginsenoside F11, two potential chemical markers present in Panax ginseng C.A.Meyer and P. quinquefolium L. methanolic extracts. GinsenosideRf and 24(R)-pseudoginsenoside F11 were identified and conformed unambiguously by accurate mass measurement and their different fragmentation pathways were performed on Q-TOF-MS. Quantitative analysis was carried out under selective ion monitoring (SIM) mode. The limit of detection (LOD) of this UPLC/Q-TOF-MS analysis for ginsenosideRf and 24(R)-pseudoginsenoside F11 was 0.05 and 0.08 ng, respectively. GinsenosideRf was linear over the range of 0.164–16.4 ng with a correlation coefficient (R2) of 0.9997, while 24(R)-pseudoginsenoside F11 was linear from 0.243 to 24.3 ng with an R2 of 0.9989. The method developed is rapid, accurate, reliable and highly sensitive for qualitative and quantitative analyses of Asian ginseng and American ginseng.

Using high-performance liquid chromatography (HPLC), a chemical fingerprint method was developed for investigating and demonstrating the variance of flavonoids among different origins of sea buckthorn berries49. Many samples were analyzed including: 15 RS (Hippophaerhamnoides ssp. sinensis) samples, 7 RY (H. rhamnoindes ssp. yunnanensis) samples, 5 RW (H. rhamnoides ssp. wolongensis) samples, 4 NS (H. neurocarpa ssp. stellatopilosa) samples and 3 TI (H. tibetana) samples. In the HPLC chromatograms, 12 compounds were identified as flavonoids. Both correlation coefficient of similarity in chromatograms and relative peak areas of characteristic compounds were calculated for quantitative expression of the HPLC fingerprints.

Worldwide traditional herbal medicines are gaining popularity as a source of complementary and alternative remedies. Herbal medicines are generally presumed as safe, harmless and without side effects, because of their natural origin. According to the source of the plant materials, herbal medicines can contain excessive or even banned pesticides, heavy metals and microbial contaminants. The presence of these contaminants as well as adulteration with conventional pharmaceuticals can lead to acute or chronic toxicity, severe side effects and drug interactions65. Several methods were developed for herbal weight loss products66-69, erectile dysfunction products70-72 or Ginkgo biloba73, 74. Ginkgo biloba extract is one of the most popular and scientifically explored pharmaceutical and nutraceutical grade raw materials that is sold world wide. The typical extract of ginkgo sold in the market is standardized to contain a minimum of 6% terpene lactones (TL), 24% flavone glycosides (FGL) and less than 5 ppm ginkgolic acids73. Chandra et al74 have investigated the typical quality of a wide variety of commercial ginkgo extracts as well as their potential adulterants. Based on our analytical results they have classified the commercially available extracts of G. biloba into three categories such as authentic, intermediate and adulterated/spiked. A combination of qualitative determination of the unhydrolyzed extracts by phytochemical fingerprinting as well as typical quantitative analysis for total flavone glycosides including Q/K/I (quercetin / kaempferol / isorhamnetin) peak area ratio on hydrolyzed samples is recommended to establish/track the authenticity of the available extracts for formulation purposes.

FTIR spectroscopy has been developed for analysis of extra virgin olive oil (EVOO) adulterated with palm oil (PO)75. Measurements were made on pure EVOO and that adulterated with varying concentrations of PO (1.0–50.0% wt./wt. in EVOO). Two multivariate calibrations, namely partial least square (PLS) and principle component regression (PCR) were optimized for constructing the calibration models, either for normal spectra or its first and second derivatives. Detection limit of adulteration was determined as 5% for corn–sunflower binary mixture, cottonseed and rapeseed oils.
The stems of Berberisaristata DC. (Berberidaceae) are used in the South Asian traditional medicine as a key ingredient in formulations for eye care, skin diseases, jaundice, rheumatism and also in diabetes. B. lyciumRoyle and B. asiaticaRoxb. are traded as equivalents of B. aristata. Conventional macro-morphology and microscopic examination does not aid in critically distinguishing the three species. DNA

markers76 were developed by amplifying and sequencing the complete internal transcribed spacer region (ITS1, 5.8S rRNA and ITS2) from the genomic DNA, using universal primers. The markers developed are efficient and reliable in authenticating B. aristata, B. asiatica and B. lycium.

5. CONCLUSIONS

Many aspects of pharmaceutical counterfeiting viz. examples, the effects, methods of detection and anti-counterfeiting measures have been discussed in this review. However, none of these would be useful without initiatives to combat the rise in the number of cases of fake and/or substandard products on the market.

The battle against counterfeit drugs has only just begun and it will be a long road ahead for those involved in getting rid of this illegal trade. The counterfeiters are becoming more technologically savvy with the consequence that it is very difficult to detect sub-standard products. However, developing technologies such as near-infrared spectroscopy, Raman spectroscopy and isotopic characterization can prove useful in providing rapid detection methods. Furthermore, by making use of sophisticated anti-counterfeiting measures such as holograms, taggants and electronic tracking, manufacturers can trace their products from production to distribution.

REFERENCES

1. http://www.who.int/medicines/organization.gsm/activities/quality_assurance/cft/counterfeit.
2. ICC Counterfeiting Intelligence Bureau, The International Anti-counterfeiting Directory, ICC Commercial Crime Services, 2008.
3. RodionovaO.Ye, Pomerantsev A.L., 'NIR-based approach to counterfeit-drug detection', ***TrAC Trends in Analytical Chemistry*** 29(8) 795-803 (2010).
4. Deisingh K., 'Pharmaceutical counterfeiting', ***Analyst***, 130: 271 – 279 (2005)
5. Wilhelmsen E.C., 'Encyclopedia of analytical chemistry; applications, theory and instrumentation '– Adulteration Determination, vol.15, 2000, pg. 3862-3888
6. Cole C., Jones L. and McVeigh J., 'Adulterants in illicit drugs: a review of empirical evidence', ***Drug Testing and Analysis***, 2011; 3(2): 89-96
7. WHO, Counterfeit Drugs: Guidelines for the Development of Measures to Combat Counterfeit Drugs, ***WHO***, Geneva, 1999.
8. www.gphf.org/web/en/start/index.htm
9. http://www.gphf.org/web_en/projekte/minilab/index.htm, 2003.
10. ICC Commercial Crime Services, http://www.iccwbo.org/ccs/news_archives/2003/counterfeit_drugs.asp, 2003.
11. Roggo Y., Degardin K. and Margot P., Identification of pharmaceutical tablets by Raman spectroscopy and chemometrics, Talanta, 2010; 81(3): 988-995
12. Martino R., Malet-Martino M., Gilard V. and Balayssac S., 'Counterfeit drugs: analytical techniques for their identification', ***Analytical &Bioanalytical Chemistry***, 2010; 398(1): 77-92
13. Bunaciu A.A., Aboul-Enein H.Y. and Fleschin S., 'Application of Fourier Transform Infrared Spectrophotometry in Pharmaceutical Drugs Analysis', ***Applied Spectroscopy Reviews*** 2010; 45(3): 206-219
14. Been F., Roggo Y., Degardin K., Esseiva P. and Margot P., 'Profiling of counterfeit medicines by vibrational spectroscopy', ***Forensic Science International***, 2011; 211(1-3): 83-100
15. Dong Y-D. and Boyd B.J., 'Applications of X-ray scattering in pharmaceutical science', ***International Journal of Pharmaceutics***, 2011; 417(1-2): 101-111
16. Dégardin K., Roggo Y., Been F. and Margot P., 'Detection and chemical profiling of medicine counterfeits by Raman spectroscopy and chemometrics', ***Anal.Chim.Acta***, 2011; 705(1-2): 334-341
17. Rodionova O. Ye., Houmøller L.P., Pomerantsev A.L., Geladi P., Burger J., Dorofeyev V.L. and Arzamastsev A.P., 'NIR spectrometry for counterfeit drug detection: A feasibility study', ***Anal.Chim.Acta***, 2005; 549(1-2): 151-158
18. Holzgrabe U. and Malet-Martino M., 'Analytical challenges in drug counterfeiting and falsification—The NMR approach'***, J. of Pharm.&Biomed.Anal***., 2011, 55(4), 679-687
19. Sharma K. and Paradakar M., 'The melamine adulteration scandal', ***Food Security***, 2010; 2(1): 97-107
20. Beni S., Limtiaco J.F.K. and Larive C. K., 'Analysis and characterization of heparin impurities', ***Analytical &Bioanalytical Chemistry***, 2011; 399(2): 527-539
21. Hu C-Q., Zou W-B., Hu W-S., Ma X-K., Yang M-Z., Zhou S-L., Sheng J-F., Yuan Li,

Cheng S-H. and Xue J., 'Establishment of a Fast Chemical Identification System for screening of counterfeit drugs of macrolide antibiotics', ***J. of Pharm.&Biomed.Anal.***, 2006; 40(1): 68-74

22. Gaudiano M.C., Di Maggio A., Antoniella E., Valvo L., Bertocchi P., Manna L., Bartolomei M., Alimonti S. and RodomonteA.L.,'An LC method for the simultaneous screening of some common counterfeit and sub-standard antibiotics: Validation and uncertainty estimation', ***J.of Pharm.&Biomed.Anal***., 2008; 48(2): 303-309

23. Sanli S., Sanli N., Gumustas M., Ozkan S.A., Karadas N. and Aboul-Enein H.Y., 'Simultaneous Estimation of Ceftazidime and Ceftizoxime in Pharmaceutical Formulations by HPLC Method', ***Chromatographia***, 2011; 74(7-8): 549-558

24. Ricci C., Eliasson C., Macleod N.A., Newton P.N., Matousek P. and Kazarian S.G., 'Characterization of genuine and fake artesunate anti-malarial tablets using Fourier transform infrared imaging and spatially offset Raman spectroscopy through blister packs', ***Anal.&Bioanal.Chem***. 2007; 389(5): 1525-1532

25. Ricci C., Nyadong L., Fernandez F.M., Newton P.N. and Kazarian S.G., 'Combined Fourier-transform infrared imaging and desorption electrospray-ionization linear ion-trap mass spectrometry for analysis of counterfeit antimalarial tablets', ***Analytical &Bioanalytical Chemistry***, 2007; 387(2): 551-559

26. Green M.D., Nettey H., Villalva Rojas Ofelia, Pamanivong C., Khounsaknalath L., Ortiz M.G., Newton P.N., Fernández F.M., Vongsack L. and OtManolin, 'Use of refractometry and colorimetry as field methods to rapidly assess antimalarial drug quality', ***J. of Pharm.& Biomed. Anal***., 2007; 43(1): 105-110

27. Dowell F.E., Maghirang Elizabeth B., Fernandez F.M., Newton P.N. and Green M.D., 'Detecting counterfeit antimalarial tablets by near-infrared spectroscopy', ***J.of Pharm.& Biomed. Anal.***, 2008; 48(3): 1011-1014

28. Panusa A. , Multari G., Incarnato G. and Gagliardi L., 'High-performance liquid chromatography analysis of anti-inflammatory pharmaceuticals with ultraviolet and electrospray-mass spectrometry detection in suspected counterfeit homeopathic medicinal products', ***J. of Pharm.& Biomed. Anal***., 2007; 43(4): 1221-1227

29. de Peinder P., Vredenbregt M.J., Visser T. and de Kaste D., 'Detection of Lipitor® counterfeits: A comparison of NIR and Raman spectroscopy in combination with chemometrics', ***J.of Pharm.& Biomed. Anal.***, 2008; 47(4-5): 688-694
30. Patient Information Sheet Viagra®, US. Department of Health and Human Services, Food and Drug Administration, RockvilleMD, USA, June 2005.

31. Lindegårdh N., Hien T.T., Farrar J., Singhasivanon P., White N.J. and Day N.P.J., 'A simple and rapid liquid chromatographic assay for evaluation of potentially counterfeit Tamiflu®', ***J. of Pharm.& Biomed. Anal***., 2006; 42(4): 430-433

32. Vredenbregt M.J., Blok-Tip L., Hoogerbrugge R., Barends D.M. and de Kaste D., 'Screening suspected counterfeit Viagra® and imitations of Viagra® with near-infrared spectroscopy', ***J. of Pharm.& Biomed. Anal***., 2006; 40: 840–849.

33. Zhou P., Oh S.S.Y., Hou P.L., Low M.Y. and Koh H.L., 'Simultaneous determination of synthetic phosphodiesterase-5 inhibitors found in a dietary supplement and pre-mixed bulk powders for dietary supplements using high-performance liquid chromatography with diode array detection and liquid chromatography–electrospray ionization tandem mass spectrometry'***, J. Chromatogr. A***, 2006; 1104: 113–122.

34. Maurin J.K., Pluciński F., Mazurek A.P. and FijałekZbigniew, 'The usefulness of simple X-ray powder diffraction analysis for counterfeit control—The Viagra® example', ***J. of Pharm.& Biomed. Anal***., 2007; 43(4): 1514-1518

35. de Veij M., Deneckere A., Vandenabeele P., de Kaste D. and Moens L., 'Detection of counterfeit Viagra® with Raman spectroscopy', ***J.of Pharm.& Biomed. Anal.,*** 2008; 46(2): 303-309

36. Sacré P.Y., Deconinck E., Saerens L., De Beer T., Courselle P., Vancauwenberghe R., Chiap P., Crommen J. and De Beer J.O., 'Detection of counterfeit Viagra® by Raman microspectroscopy imaging and multivariate analysis', ***J.of Pharm.& Biomed. Anal***., 2011; 56(2): 454-461

37. Sacré P.Y., Deconinck E., De Beer T., Courselle P., Vancauwenberghe R., Chiap Patrice, Crommen J. and De Beer J.O., 'Comparison and combination of spectroscopic techniques for the detection of

counterfeit medicines', J.*of Pharm.& Biomed. Anal*., 2010; 53(3): 445-453

38. Xie L-G., Sun H-M. and Jin S-H., 'Screening adulteration of polypropylene bottles with postconsumer recycled plastics for oral drug package by near-infrared spectroscopy', ***Anal.Chim.Acta***, 2011; 706(2): 312-320

39. Causin V., Marega C., Carresi P., Schiavone S. and Marigo A., 'A quantitative differentiation method for plastic bags by infrared spectroscopy, thickness measurement and differential scanning calorimetry for tracing the source of illegal drugs', ***Forensic Science International***, 2006; 164(2-3): 148-154

40. de Carvalho L.M., Martini M., Moreira P. A. L., de Lima A.P.S., Correia Daniele, Falcão Tatiana, Garcia Solange C., de Bairros A.V., do Nascimento P.C. and Bohrer Denise, Presence of synthetic pharmaceuticals as adulterants in slimming phytotherapeutic formulations and their analytical determination, ***Forensic Science International***, 2011; 204(1-3): 6-12

41. Zou H-B., Yang G-S., Qin Z.Q. , Jiang W.Q., Ai Qin Du and AboulEnein H.Y., Progress in Quality Control of Herbal Medicine with IR Fingerprint Spectra, ***Anal.Lett***., 2005; 38(9): 1457-1475

42. Bunaciu A.A., Aboul-Enein H.Y. and Fleschin S., Recent Applications of Fourier Transform Infrared Spectrophotometry in Herbal Medicine Analysis, ***Applied Spectroscopy Reviews*** 2011; 46(4): 251-260

43. Yap K.Y-L.., Chan S.Y. and Lim C.S., Infrared-based protocol for the identification and categorization of ginseng and its products, ***Food Research International***, 2007: 40(5): 643-652

44. Vardin H., Abdullatif T., OzenB.andMauer L., Authentication of pomegranate juice concentrate using FTIR spectroscopy and chemometrics, ***Food Chemistry***, 2008; 108(2): 742-748

45. Zhang L. and Nie L., Discrimination of geographical origin and adulteration of radix astragali using fourier transform infrared spectroscopy and chemometric methods, ***Phytochemical Analysis*** 2010; 21(6): 609–615

46. Zou P., Hong Y. and Koh H.L., Chemical fingerprinting of Isatisindigotica root by RP-HPLC and hierarchical clustering analysis, ***J. of Pharm.& Biomed. Anal***., 2005; 38(3): 514-520

47. Xie P., Chen S., Liang Y-Z., Wang X., Tian R. and Upton R., Chromatographic fingerprint analysis—a rational approach for quality assessment of traditional Chinese herbal medicine, ***J.of Chromatography A***, 2006;1112(1-2): 171-180

48. Xie G. X., Qiu M. F., Zhao A. H. and Jia W., Fingerprint Analysis of FlosCarthami by Pressurized CEC and LC, ***Chromatographia***, 2006; 64(11-12): 739-743

49. Chen C., Zhang H., Xiao W., Yong Z-P. and Bai N., High-performance liquid chromatographic fingerprint analysis for different origins of sea buckthorn berries, ***J. of Chromatography A,*** 2007; 1154(1-2): 250-259

50. Liang Y., Xie P. and Chau F., Chromatographic fingerprinting and related chemometric techniques for quality control of traditional Chinese medicines, J. of Separation Science 2010; 33(3): 410–421

51. Qiao X., Han J., He W-N., Wu T., Wang B-R., Ye M. and Guo D-A., 'Chemical fingerprint of commercial Radix Echinopsis and quantitative analysis of α-terthienyl', ***J.of Separation Science*** 2010; 33(4-5): 530–538

52. Hu L.F., Li S.P., Cao H., Liu J.J., Gao J.L., Yang F.Q. and Wang Y.T., 'GC–MS fingerprint of Pogostemoncablin in China', ***J. of Pharm.& Biomed. Anal***., 2006; 42(2): 200-206

53. Marchei E., Pichini S., Pacifici R., Pellegrini M. and Zuccaro P., 'A rapid and simple procedure for the determination of synephrine in dietary supplements by gas chromatography-mass spectrometry', ***J. of Pharm.&Biomed.Anal***., 2006; 41(4): 1468-1472

54. Lucio-Gutiérrez J.R., Coello J. and Maspoch S., 'Application of near infrared spectral fingerprinting and pattern recognition techniques for fast identification of Eleutherococcussenticosus', Food Research ***International***, 2011; 44(2): 557-565

55. Nicoletti M. and Petitto V., 'Contaminations of herbal products determined by NMR fingerprint', ***Natural Product Research***, 2010; 24(14): 1325-1329

56. Sombra L. L., Gómez M. R., Olsina R., Martínez L. D. and Silva M. F., 'Comparative study between capillary electrophoresis and high performance liquid chromatography in 'guarana' based phytopharmaceuticals',***J.of***

Pharm. & Biomed. Anal ., 2005; 36(5): 989-994

57. Sze S. C-W., Song J-X., Wong R-N-S., Feng Y-B., Ng T-B.,Tong Y. and Zhang K.Y-B., 'Application of SCAR (sequence characterized amplified region) analysis to authenticate Lyciumbarbarum (wolfberry) and its adulterants', ***Biotechnology & Applied Biochemistry***, 2008; 51(1): 15–21

58. Kiran U., Khan S., Mirza K.J., Ram M. and Abdin M.Z., SCAR markers: 'A potential tool for authentication of herbal drugs', ***Fitoterapia***, 2010; 81(8): 969-976

59. Li M., Jiang R-W., Hon P-M., Cheng L., Li L-L., Zhou J-R., Shaw P-C. and But P.P-H., 'Authentication of the anti-tumor herb Baihuasheshecao with bioactive marker compounds and molecular sequences', ***Food Chemistry***, 2010; 119(3): 1239-1245

60. Zou P., Hong Y. and Koh H.L., 'Chemical fingerprinting of Isatisindigotica root by RP-HPLC and hierarchical clustering analysis', ***J. of Pharm.&Biomed.Anal***., 2005; 38(3): 514-520

61. Li W., and Fitzloff, J.F., 'HPLC analysis of ginsenosides in the roots of Asian ginseng (Panax ginseng) and North American ginseng (Panaxquinquefolius) with in-line photodiode array and evaporative light scattering detection'. ***J. of Liquid Chromatography and Related Technologies***, 2002; 25(1): 29–41.

62. Kevin Y-L.Y., Chan S.Y. and Chu Lim C.S., Authentication of traditional Chinese medicine using infrared spectroscopy: distinguishing between ginseng and its morphological fakes, ***J. of Biomedical Science***, 2007; 14(2): 265-273

63. Edwards H.G.M., Munshi T. and Page K., Analytical discrimination between sources of ginseng using Raman spectroscopy, ***Analytical &Bioanalytical Chemistry***, 2007; 389(7-8): 2203-2215

64. Li Li, Guo-An Luo, Qiong-Lin Liang, Ping Hu and Yi-Ming Wang, Rapid qualitative and quantitative analyses of Asian ginseng in adulterated American ginseng preparations by UPLC/Q-TOF-MS, ***J.of Pharm.& Biomed. Anal.,*** 2010; 52(1): 66-72

65. Nelson L., Shih R. and Hoffman R., Aplastic anemia induced by adulterated herbal medication, ***J. Toxicol. Clin. Toxicol***. 1995; 33: 467–470.

66. de Carvalho L.M., Martini M., Moreira A. P., Garcia S. C., do Nascimento P.C. and Denise B., Determination of synthetic pharmaceuticals in phytotherapeutics by capillary zone electrophoresis with contactless conductivity detection (CZE-C4D), ***Microchemical Journal***, 2010; 96(1): 114-119

67. VermaakI., Hamman J.H. and Viljoen A.M., High performance thin layer chromatography as a method to authenticate Hoodia gordonii raw material and products, ***South African Journal of Botany***, 2010; 76(1): 119-124

68. Wang J., Chen B. and Yao S., Analysis of six synthetic adulterants in herbal weight-reducing dietary supplements by LC electrospray ionization-MS, ***Food Additives & Contaminants: Part A***, 2008; 25(7): 822-830

69. Zhang J., Zhang X., Dediu L., Cristea V., Review of the current application of fingerprinting allowing detection of food adulteration and fraud in China, ***Food Control***, 2011; 22(8): 1126-1135

70. Reepmeyer J.C. and Woodruff J.T., Use of liquid chromatography–mass spectrometry and a hydrolytic technique for the detection and structure elucidation of a novel synthetic vardenafil designer drug added illegally to a "natural" herbal dietary supplement, ***J.of Chromatography A***, 2006; 1125(1): 67-75

71. Gryniewicz C. M., Reepmeyer J.C., Kauffman J.F. and Buhse L. F., 'Detection of undeclared erectile dysfunction drugs and analogues in dietary supplements by ion mobility spectrometry2, ***J.of Pharm.& Biomed. Anal.***, 2009; 49(3): 601-606

72. Singh S., Prasad B. , SavaliyaA.A.,Shah R.P.,. Gohil V.M. and Kaur A., Strategies for characterizing sildenafil, vardenafil, tadalafil and their analogues in herbal dietary supplements, and detecting counterfeit products containing these drugs, ***TrAC Trends in Analytical Chemistry***, 2009, 28(1), 13-28

73. Bombardelli, E., Mustich, G., and Bertani, M. (1989). New extracts of Ginkgo biloba and their methods of preparation. ***European patent*** 89309500.0 – A61K 35/78.

74. Chandra A., Li Y., Rana J., Persons K., Hyun C., Shen S. and Mulder T., Qualitative categorization of supplement grade Ginkgo biloba leaf extracts for authenticity, ***J. of Functional Foods,*** 2011; 3(2): 107-114

75. Rohman A. and Che Man Y.B., Fourier transform infrared (FTIR) spectroscopy for analysis of extra virgin olive oil adulterated with palm oil, ***Food Research International,*** 2010; 43(3): 886-892

76. Balasubramani S.P. , Goraya G.S. and Venkatasubramanian P., Development of ITS sequence-based markers to distinguish Berberisaristata DC. from B. lyciumRoyle and B. asiaticaRoxb., ***3 Biotech***, 2011; 1(1): 11-19