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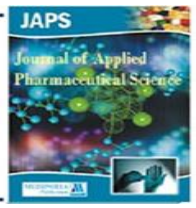

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Recent analytical approaches to counterfeit drug detection

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ABSTRACT

Counterfeiting of drugs has now become a lucrative multi-billion dollar business that threatens the effective delivery of health care services. Apart from Africa and other marginally developed parts of the world, the developed economies are equally not spared from the bane of this trade. To combat this menace, concerted efforts are needed from various facets of the community. In addition to visual inspections, sophisticated analytical tools are usually required in order to successfully distinguish counterfeits from the real stuffs. Attempt is made in this review to summarize the recent applications of these tools in keeping track with counterfeit drug flow. These are discussed under the headings of different approaches, including Near Infrared Spectroscopy, Raman Spectroscopy, Fluorescence and Phosphorescence measurements, Nuclear Magnetic Resonance imaging, X-ray and radio frequency analysis. In the quest to maintain their profit margin, the perpetrators of counterfeit pharmaceuticals will continue to develop ingenious ways to circumvent regulatory systems. Therefore, relentless efforts should continue to be deployed toward research and development in low cost, fast and efficient ways of detecting counterfeit drug at different stages of its existence.

Key words: Counterfeit drugs, analytical tools, near infrared spectroscopy, nuclear magnetic resonance imaging, radio frequency analysis, detection.

INTRODUCTION

Antimicrobials such as Cinchoma bark extract and quinine have been faked as early as the 1600s (Newton et al., 2010). To better understand this topic, pnfiroper distinction should be made between counterfeit drugs (CDs) and substandard drugs: CDs are any drug products that have been criminally and intentionally mislabeled with regard to its identity or source. Examples include fake packaging, absence of active principles or their presence at incongruous amounts (Caudron et al., 2008). On the other hand, substandard drugs are genuine products that do not meet the specified quality set for them due to carelessness, incompetence, obsolete or malfunctioning equipment. Reasons for indulging in and patronizing CDs include: ease of access and availability, economic, confidentially and crave for easy and large profits (Casola et al., 2009; Burki, 2010). CDs can be found on display in shops alongside the real stuff, but at considerably cheaper price- a case of one standard for the rich and another for the poor. Some countries in Africa can have as high as 30% of the drugs in market as CDs. These mostly flow from India and China (Costa, 2010) with the trade expected to reach US$ 75 billion by 2010.

The most apparent impacts of CDs are death and resistance. About 500 children were reportedly killed by taking paracetamol that has been contaminated with a renal toxin (Newton et al., 2006). Widespread use of counterfeit and substandard drugs can lead to drug resistance in microbes. An example is the resistance to Artemisinin, a first-line of treatment for uncomplicated *Plasmodium falciparum* malaria, in Cambodia-Thailand border (Dondorp et al., 2010). Other

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impacts of CDs include the possibility of contamination with traces of the ecstasy drug 3, 4-Methylenedioxymethamphetamine (MDMA) in medicines coming from the same pressing tools, and financial loss to genuine manufacturers since the CDs are mostly cheaper than the genuine ones.

The internet is a big source of concern in the fight against counterfeiting of drugs. Drugs can now be found on display on the eBay and other internet sites (Berman, 2008). Since dealing in counterfeit drugs can bring easy and quick money, concerted effort by various facets of society is needed to keep this illicit trade under check; pharmaceutical companies in association with medical regulatory authorities should put in place system of tracking genuine products from source to the point of delivery, and make sure that only medicaments approved are being sold to the public. This effort should adequately be supported by the politicians who must provide the needed statutes for safeguarding the populace against effect of dangerous materials, including CDs.

Most of the time, one needs to go a little further beyond physical examination in order to tell apart counterfeit from the genuine stuff. Various analytical methods can be found that are applicable for the detection of counterfeit drugs. While some of these methods use non-invasive tools that can detect drugs even in their capsules or ampoules, others involve chemical analysis capable of detecting the active ingredients and impurities in drug preparations. By applying Raman mapping with multivariate image segmentation using a spatial directional agglomeration clustering method (Lin et al., 2006), the chemical homogeneity of drugs can be established right from production lines. This would help in reducing the level of substandard formulations in circulation and consequently the level of false positives during analysis. Because of their effort to avoid detection, counterfeiters can include a small percentage of active ingredients in their illegal preparations. Therefore, detection should not only be qualitative but also quantitative. The ideal analytical tool or method for detecting counterfeit medicines should be of low cost and fast, and should have high level of reliability. It should avoid error in classifying counterfeit drugs as genuine and vice-versa.

NEAR INFRARED (NIR) APPROACH

Absorption bands corresponding to mid-IR region (4000-2500 cm^{-1}) are used for the identification of compounds (Blanco and Villarroya, 2002). Near infrared spectroscopy (NIRS) has found applications even in the pharmaceutical sector (Boiret et al., 2011). According to Rodionova and Pomerantsev (2010), NIRS possesses the following advantages: Rapid and simple, because it involves minimal or no sample prep; very informative spectra which give information on both physical and chemical phenomena; different modes can be used depending on the type of sample-transmittance, diffuse reflectance and scattering modes; offers on-site analysis through the use of fiber optic probes. In one application using this principle, detection of counterfeit anti-malarial tablets was accomplished (Dowell et al., 2008). 62 genuine artesunate anti-malarial tablets and 55 counterfeit tablets obtained from South-East Asia were analyzed. Half tablet each was used for NIRS and results were statistically analyzed without apriori knowledge to prevent bias in classification of the drugs. Identification was based on the strong absorbance of artesunate around 1200, 1360, 1700 and 2300 nm corresponding to various C-H vibrational modes in the structure. Smooth edge of each tablet was placed over a 4 mm diameter opening on a brass fixture in the QaulitySpec Pro Spectrometer. This uses a HL2000 halogen lamp source for illumination, 78 fibers each for illumination and collection of reflected light (300-2500 nm region). Spectra were collected using 6.3 mm diameter bifurcated probes. Measurement of visible and NIR radiation was performed with silicon and indium-gallium-arsenide sensors. 20 spectra were collected and averaged for each tablet. The procedure took only 1 min per tablet. Based on their NIRS spectra, all the anti-malarial drugs were identified as genuine or counterfeit with 100% accuracy using US $ 45000 portable and user friendly NIRS tool.

For the first time, the diastereomer of tadalafil was identified in counterfeited pharmaceuticals with the aid of NIR (Venhuis et al., 2010). Although the trans-tadalafil has some phosphodiesterase 5 (PDE-5) inhibitory activities, the optical purity of dalalafil should be ascertained to avoid possible injurious health implications since data on the toxicity profile of this stereo-form is lacking. In a different application (Lopes et al., 2009), classical least square analysis (CLSA) was used to analyze the results obtained from near infrared chemical imaging (NIR-CI) of Heptodin tablets. A plus for this method is the fact that no prior information about the origin of the counterfeit drug is necessary in order to obtain good result. In this study, 12 counterfeit tablets were resolved with good precision using concentration maps of the pure API to compare with those of the counterfeits. Clear difference was seen between tablets with high contents of the API, lamivudine, and those with low content. The importance of using statistical analysis tools to interpret the tons of data obtained through NRI-CI was further stressed in this study. In addition, a recently developed statistical image analysis method, named by its authors as symmetry parameter image analysis (SPIA), was explained for the analysis of data obtained from NIR-CI measurements (Puchert et al., 2011). As already expatiated by this research group (Puchert et al., 2010), identification of counterfeit drug may follow a four-stage approach: Visual characterization, NIR/NIRS-CI (PCA), NIR-CI analysis(PLS) which is more detailed form of the PCA, and finally variance analysis in which an image signature is used to characterize variability. Through the symmetry of pixel distributions, the attributes of components in drug preparations, in this case counterfeit drugs, can be determined and compared to the original. This method is more objective, and will help in minimizing errors of interpretation that abound in the highly subjective approach of visual examination of inhomogeneity arising from differences in mode of drug preparation. A review on the use of chemometrics and NIR spectroscopy in pharmaceutical applications was previously prepared (Roggo et al., 2007). Apart from checking the homogeneity of a batch, the NIRS based chemometric method described for the fast screening of Viagra can

also distinguish between counterfeited and imitated Viagra and the real stuff through the detection of sildenafil citrate, the API in Viagra formulations (Vredenbregt et al., 2006). One of the demerits of such predictive methods is the high possibility of false positives. However, with high coefficients of correlation (>0.99), this method was able to correctly and precisely predict the absence or presence of this active ingredient in at least 98% of the analyzed 103 samples of different origins and compositions.

RAMAN SPECTROMETRIC MEASUREMENT APPROACH

Since the discovery of Raman Effect, a type of scattering, in 1928, its various applications have continued to attract interest (Agarwal and Atalla, 1995). In Raman Spectroscopy, a laser shone on a sample is scattered and this scattering leads to two principal types of processes known as stokes and anti-stoke: In the stokes process, which is parallel to absorption, scattered photons are shifted to lower frequency due to abstraction of vibrational energy by the analyte molecules. For the anti-stoke process, parallel of emission, scattered photons are shifted to higher frequency arising from picking up energy released from molecules. These effects are displayed as Raman Shift in a typical Raman spectrum. When a Rayleigh scattering, which is more intense than Raman scattering, occurs due to optical heterogeneities or density variations, the scattering photons are not shifted in frequency. It has now become possible, using the potentials of nanotechnology, to amplify the scattering phenomenon in Raman via surface-enhanced Raman Spectroscopy (SERS) (Golightly et al., 2009). A review on the various forensic and homeland security applications of Raman Spectroscopy has been compiled (Izake, 2010). Raman is complementary to many techniques. More anti-malarial drugs bought covertly in Ghana and Nigeria has failed quality control checks using Raman Spectrometric measurements than with physical examination (Bate and Hess, 2010). Unlike the case of NIRS where long time exposure of drugs can lead to absorption of water from the atmosphere which affects the NIRS procedure and results, Raman has inherently low vibrational activities due to water (de Peinder et al., 2008). This is an important advantage as the shelf life of many drugs is long and these drugs are often subjected to extreme environmental conditions within this period. As a non-destructive technique, the method was successfully applied to the determination of thickness of tablet coating under different coating conditions (Cahyadi et al., 2010). A rugged, hand-held instrument based on Dispersive Raman Spectroscopy (DRS) has been developed (Bugay and Brush, 2010). This instrument can be used remotely and methods developed in one instrument can be transferred to another for application in tracking counterfeited drug preparations.

Raman microscopic evaluation of technology dependent structural differences in tablets containing imipramine model drug was explained (Vajna et al., 2010). The model tablets contained imipramine hydrochloride as API and the excipients were microcrystalline cellulose, maize starch, hydroxypropyl-methylcellulose as binder and magnesium stearate as lubricant. Seven different manufacturing methods were employed and coded as D, HS-W, HS-A, F-W, F-H, F-A and F-AH. Mechanical properties of the prepared tablets were tested with PharmaTest PTB-311 device, by carrying out measurements of 10 tablets for each compression force level in each batch. Raman-mapping spectra were collected with the aid of a Horiba Jobin-Yvon LabRAM system that was coupled with an external 785 nm diode laser source and an Olympus BX-40 optical microscope. For optical imaging and spectral acquisition, objectives of 10× and 100× were used. As the laser beam was directed through this objective, backscattered radiation was collected with same objective. The collected radiation was then filtered to remove Rayleigh photons by directing it through a notch. It was then channeled through a confocal hole and the entrance slit onto a grating monochromator containing 950 grooves/mm and this dispersed the light before it got to the CCD detector. The spectrograph was set to provide a spectral range of 400-1630 cm^{-1} at 2 cm^{-1} resolution. Raman spectra of ingredients were taken at acquisition time of 100 s. In order to maintain the original sample identity, no sample prep was employed. The API band at 1597 cm^{-1} was reliably used for the determination of the API content with Direct Classical Least Square (DCLS) modeling. Fractures of D and F-W tablets occur at higher forces compared to HS-W tablets, at all compaction forces used in the manufacturing process. The higher percentage of well-dispersed lubricant HS-W process has led to decrease in cohesion among the particles resulting in lower tablet strength compared to those of other processes. Since results obtained indicate that different manufacturing technology may result in polymorphism, morphological variations may help to trace the technology used for production. One cause of change in morphology of API is the presence of moisture in the course of the process. When ingredients are compressed directly without involving any wet granulation step, the spectrum of the imipramine particles showed same morphology as found in the original API. RSD of <9% in HS granulation and >17% in any other batch is because API upon drying forms a homogeneous layer on the particles of the excipients. Therefore, the smaller the RSD value the more homogeneous the particle distribution in the tablet.

Similarly, 50 apparent artesunate anti-malarial tablets that were purchased from SE Asia were analyzed with Raman Spectrometer. This instrument detected only minimal amount of the API in all these drugs, while most of the tablets were awash with various excipients, paracetamol and even titanium dioxide (TiO_2). These results were further confirmed using colorimetric as well as chromatographic approaches. The presence of TiO_2 in the surface of capsule can hamper the signal of backscattering Raman due to relatively lower intensity of the internal components as compared to such strong signals from TiO_2. A method that was able to bias against such occurrence has been developed (Eliasson et al., 2008). In this procedure, transmission Raman geometry was employed to obtain a suppression factor of 33 in the signal of such strong scattering surface layer component relative to that of the internal content of the capsules. Another type of Raman that can

achieve this result is known as Spatially Offset Raman Spectroscopy (SORS). Nurofen caplets and Lloyd's capsules both contain ibuprofen as the API. These preparations may also contain paracetamol as one of the excipients. Paracetamol, a higher Raman scatterer than ibuprofen poses a great challenge in the analysis of this active ingredient. However, through the use of SORS, positive identification was readily obtained (Eliasson and Matousek, 2007).

FLUORESCENCE AND PHOSPHORESCENCE APPROACHES

Camptothecin (CPT) - analogs are commonly used as anticancer drugs due to their selective inhibition of an enzyme involved with DNA transcription. However, due to their high cost, these drugs are frequently a subject of counterfeiting through the use of CPT which has low water solubility and contains toxic effects. Recently, a method based on room temperature phosphorimetry was employed in the determination of CPT as trace contaminant in anticancer formulations containing irinotecan (CPT-11) or topotecan (TPT) as active ingredients (Maia et al., 2010). Previously, a fast and low cost method based on light-induced fluorescence was developed for the analysis of pharmaceutical ingredients (Lai et al., 2004). While some compounds are fluorescent in nature and are, therefore, suitable for analysis per se using this approach, derivatization may be necessary for others in order to produce an adduct that is fluorescent. An example of this is the reaction between non-fluorescent oseltamivir phosphate with fluorescamine to give an adduct that is quantifiable (limit of quantitation in parts par million) at 483 nm using excitation of 381 nm (Aydogmus, 2009). Elsewhere, laser-induced fluorescence with photochemical derivatization was used for the trace determination of CPT in anticancer drug preparations (de C Marquesa et al., 2010). A related approach is the use of small array to discriminate between functional groups in drugs. A very recent method in this regard was published (Davey et al., 2011). The arrays were obtained from three reactive cruciform fluorophores in six different solvents, and were able to differentiate between 10 different carboxylic acids. For selectivity in this approach, highly selective fluorescent tags can now be designed. These molecular sensors are attached to target compounds in order to aid in their screening, and have been applied in forensic arena (Valeur, 2008). Based on the sensor molecule Polymer-H and on heparinase I inhibition by oversulfated chondroitin sulfate (OSCS), a method for the detection of heparin falsification has been forwarded (Alban et al., 2011). When a heparin sample is incubated with heparinase I, the resultant fluorescence intensity can be measured and used for the quantitative determination of heparin. While most of these methods are based on the properties of the bulk compounds, a method was developed that was able to establish the elemental profiles of pharmaceutical preparations in addition to coating thickness measurement (Mazel et al., 2011). This non-destructive approach is based on 3D micro-X-ray analysis.

A sensitive method based on Light Induced Fluorescence (LIF) was proposed for determining the API contents in tablets (Domike et al., 2010). This method of surface measurement for predicting total content fluorescent API of tablets was used for two different sets of ingredients. One set contained triamterene as API with lactose and colloidal silicon dioxide as excipients. The tablets, in four batches, were produced in Carver hydraulic tablet press; the first three batches contained 1.64%, 3.22% and 4.75% by weight of triamterene respectively while the fourth contained powder blends of the first three without additional mixing to make it less homogeneous. The average mass of these tablets was 320 mg. The second set contained caffeine as API and microcrystalline cellulose, lactose and magnesium stearate as excipients. These were produced in three batches made on 16 station beta press with three ton compression loads. They contained 5%, 10% and 20% by mass of the API respectively. The average weight of the tablets was 500 mg. UV absorbance was compared to LIF of the tablets. The filter used in the LIF instrument, an XF22, was set at $\lambda_{ex/em}$ of 485 nm (bandwidth 22 nm)/530 nm (bandwidth 30 nm) for triamterene and none of the other tablet ingredients was significantly fluorescent near this excitation wavelength of triamterene. Four different strategies were tested for sampling the tablets at different positions using a rotating dish on the LIP instrument. Linear sample mode was found to be more accurate compared to others. Each triamterene tablet was dissolved in 10 mL of formic acid, filtered to remove un-dissolved excipients, and the resulting solution diluted with 100 mL of 10% formic acid in water. Another dilution of 10 mL solution was done using 400 mL of 10% formic acid in water. Three 100 µL wells were filled with each of these diluted samples, in the 96-well plate used. A microplate UV reader set at 340 nm was used to monitor each well. For caffeine tablets, a weighed mass of each tablet was dissolved in 200 mL of deionized water, shaken and left overnight to settle the un-dissolved excipients. Next day, 70 µL from the clear supernatant was diluted with 930 mL of water and pipetted into a quartz vial. Quantification of caffeine was carried out by measuring absorbance at 280 nm using a UV reader of a Hewlett Packard 8452A Diode Array Spectrophotometer. It was shown that using LIF, API can be correctly estimated in 88% tablets within 10% error of UV determination. From this population statistics, it was found that the worst reproducibility was obtained in the unmixed, blended triamterene batch, as a result of inhomogeneity of the surface property.

NUCLEAR MAGNETIC RESONANCE APPROACH

As early as 1963, quantitative NMR was applied with good reproducibility to the determination of various drugs in reasonable time (Hollis, 1963). Where many techniques fail, NMR can give useful information for resolving the structures of closely related compounds. Principles and challenges of the different NMR methods for studying spectra of complex mixtures can be found elsewhere (Novoa-Carballal et al., 2011; Holzgrabe and Malet-

Martino, 2011), and a comprehensive review on techniques and recent applications of quantitative NMR spectroscopy has been written by Holzgrabe (2010). Characterization of a novel analogue of vardenafil included as an adulterant in a dietary supplement (MEGATON) was carried out using NMR approach (Lee et al., 2011). ^{1}H and ^{13}C NMR were performed in addition to HPLC and IR to establish the structure of a novel analogue of vardenafil found in the dietary supplement MEGATON which was imported to the USA from South Korea. Content of this supplement includes brewer's yeast (65%), vitamin C (15%) and dried Ganoderma lucidum Krast powder (8.25%). Vardenafil hydrochloride and hongdenafil (acetildenafil) citrate were obtained from the Korea Food and Drug Administration. 12.593 g sample was extracted twice with 75 mL of methanol/water (70:30) each. 1% sodium bicarbonate was used to adjust the pH to 9 or 10 and the solution was extracted with 75 mL dichloromethane three times. At 35^{o}C, the dichloromethane layer was evaporated to dryness and the residue re-dissolved in 100 mL of 50% methanol. The pH was adjusted to 1 or 2 with HCl. Dichloromethane was used to remove impurities in this solution twice and the pH of the methanol layer was adjusted to above 9 with the 1% sodium bicarbonate. This was extracted again with 75 mL of dichloromethane twice and then evaporated to dryness at 30^{o}C. 20 mg of the residue was dissolved in 0.75 mL deuterated chloroform ($CDCl_3$). To acquire the carbon and proton NMR spectra, Bruker AVANCE 600 Spectrometer, 14.1 T, was used. ^{1}H and ^{13}C NMR spectra of the compound under investigation were obtained and these were used to propose a structure for it which closely resembles that of vardenafil, with the only difference being the sulfonyl group in the latter substituted with an acetyl one in the former. The compound was identified as 2-(2-ethoxy-5-(2-(4-ethylpiperazin-1-yl)acetyl)phenyl)-5-methyl-7-propyl-imidazo(5,1-f)-(1,2,4) triazin-4(3H)-one, also called acetylvardenafil.

To complement ^{2}H NMR, quantitative ^{13}C NMR was also employed for the investigation of counterfeiting in drug preparations (Silvestre et al., 2009). In this application, site-specific mononuclear ^{13}C profiles were established for two active ingredients, paracetamol and aspirin. Prior to the introduction of the method of quantitative adiabatic ^{13}C NMR pulse sequences for site-specific isotopic measurements (Thibaudeau et al., 2010), fingerprinting of pharmaceuticals via the determination of $^{13}C/^{12}C$ site-specific isotope ratios has suffered from lack of good sensitivity and usually took longer time to completion as was the case in the preceding example of paracetamol and aspirin. These short-comings have now been addressed with the advent of the said method. Its application to ibuprofen has yielded a precise site-specific ^{13}C isotope profile. This was partly achieved through implementation of 180^{o} adiabatic composite refocusing pulses with improved refocusing of the chemical shift of ^{13}C along pulse sequence. Through this approach, repeatability was greatly enhanced. A poly nuclear NMR may be more complex and requires complex statistical analysis for the tons of data obtained. However, when well grasped, this method may prove very vital in the determination of counterfeit drugs of various origins. Wawer et al (2005) have applied a poly-nuclear NMR consisting of ^{1}H, ^{13}C and ^{15}N for the analysis of sildenafil base and citrate in solution, solid state and pharmaceutical dosage forms.

X-RAY DIFFRACTION AND RADIO FREQUENCY MEASUREMENT APPROACHES

X-ray diffraction (XRD) and radio frequency measurements can serve individually or as complements of Raman and IR spectroscopy in the determination of counterfeit items of pharmaceutical importance. The operational principles of using diffraction patterns in the study of different materials have recently been explained in details (Dong and Boyd, 2011). Recently, XRD was employed in the identification of rimonabant polymorphs, sibutramine and their analogs present in counterfeit Acomplia products bought through the internet (Venhuis et al., 2011). In a published study, magnetically molecularly imprinted polymers (MMIPs) were produced as a core shell and used for the extraction and sample clean-up of sildenafil due to the high recognition ability and high binding kinetics of these MMIPs for sildernafil (Ding et al., 2011). The identities of these polymers were confirmed using XRD among other analytical techniques. A new technique resembling DNA fingerprinting is being developed for the authentication of items including pharmaceutical products (Kwok et al., 2010). The working principle of physimetric identification (Physi-ID) was explained for the physical identification of objects (Kwok et al., 2011). This works like the biometric identification by fingerprinting. A physical feature of an object (e.g., drug) is given a unique ID. When these two match, an alert free situation indicative of a pass will result. Otherwise, a fail will result in an alert at Physi-ID check station, which can mean either lack of physical feature or object does not have a matching auto-ID. Physical properties such as Raman spectrum, texture feature, dimensions, weight, color pattern**,** shape, and light reflective/ refractive index will be read at the check station with the aid of a radio frequency identification (RFID) technology. Acierno et al (2010) have tried to assuage some fears with regard to possible alteration in the molecular structures of drugs due to exposure to doses of electromagnetic radiation. In this study, even after 72 h exposure of insulin to electromagnetic field from RFID tracing systems, no differences between the control and test samples were noticed, as confirmed from experiments using both NMR and HPLC methods.

One of the drawbacks of the RFID technology is the possible cloning of RFID tags. To prevent financial loss, among other negative consequences due to cloning, mechanisms for detecting clones should be put in place by RFID-chip manufacturers. Some of the ways by which clones can be detected have been compiled in a recent essay (Lehtonen et al., 2009). Recently, a hardware-enabled technology has been developed which would enable the creation of physically-unique RFID tags and make them nearly impossible to exactly clone (Lakafosis et al.,

2011). This certificate of authenticity method serves as a fingerprinting mechanism that utilizes random 3D scattering structures which are unique for individual compounds in drugs.

MISCELLANEOUS APPROACHES

In addition to the various techniques established above for tracking counterfeited items of pharmaceutical origin, there are numerous other methods that are employed for this end. For instance, because of the importance of nanotechnology to drug manufacture and delivery, nano-patterned features can now be created for drugs to serve as watermarks for these preparations (Lal et al., 2010). Multidimensional atomic force microscopy (m-AFM) can then be employed to track these patterns as a way of fighting counterfeits. Generic analytical methods already in the field are still very vital in this prolonged fight against drug adulteration and counterfeiting. A liquid chromatographic method coupled with two-dimensional mass spectrometry has been applied with good precision in the tracking of synthetic hypoglycemic drugs added to anti-diabetic formulations (Pang et al., 2009). Elimination of sample prep, shortening of analysis time and the ability of carrying out the analysis in the open air without the need for high pressure vacuums are some of the advantages that can be obtained using Fourier-transform infrared imaging in combination with electrospray ionization linear ion trap mass spectrometry. This method was recently applied for the analysis of counterfeit anti-malarial tablets with high sensitivity of detection (Ricci et al., 2007). To detect trace levels of drugs with high rapidity and sensitivity, ion mobility spectrometry and direct analysis in real time (DART) spectrometry can be used. In one application, the protonated dimer ions of AG-013736 in the anti-tumor drug Axitinib were used for the identification of the active ingredient within the analysis time of only 5 s (Likar et al., 2011). The efficiency of ionization mechanisms greatly affects the performance of mass spectrometric techniques. One technique that has the ability to efficiently ionize both polar and non polar analytes is called desorption atmospheric pressure photo-ionization (DAPPI) in which sufficiently high MS spectrum could still be obtained from drugs placed afar from MS extension inlet than conventionally practiced (Kauppila et al., 2011). This has an added advantage of avoiding contamination of the MS inlet.

Since the time capillary electrophoresis (CE) became popular in the 1980s, different applications can now be found in the pharmaceutical arena (Morzunova, 2006). It has recently been applied for the quantitative determination of insulin in insulin formulations (Staub et al., 2010). Not long ago, a reliable and low-cost type was developed for drug quality control and for the detection of counterfeit medicines (Marini et al., 2010). Rodionova et al. (2010) have recently applied a non-invasive detection method for the analysis of ampoules of dexamethasone via NIR. The performance of this approach was appraised through CE-UV confirmation of injectable dexamethasone counterfeited ampoules, in addition to GC-MS and HPLC-DAD systems. In this study, two genuine batches (G1 and G2) and a batch of counterfeit (F2) in 1 mL closed transparent glass ampoules of 4% dexamethasone 21-phosphate were used. Each batch contained a total of 15 ampoules. CE was performed using a Capel 105M electrophoresis system connected to a Mulitchrom 1.56 data acquisition system. A standard 60-cm long fused silica capillary with 75 μm i.d. and a detection window of 50 cm was employed. The system was buffered with a solution containing 10 mM sodium tetraborate and 40 mM of sodium dodecyl sulphate. The set-up was worked at 25 kV and 25^{o}C. Hydrodynamic injection mode (30 mbar, 5s) was used and the samples were analyzed at 254 nm. Production time and series labels on batches G2 and F2 were identical. A secret printing on G2 pack was used to tell the two apart. The difference between batch G1 and F2 was resolved with the CE experiment. At least three impurities were found in the fake ampoules (F2) which were absent from the genuine ones. This shows the reliability of CE which can discriminate between compounds that are similar in structures.

CONCLUSION AND FUTURE DIRECTION

As counterfeiting is a multibillion dollar business, albeit heinous, the perpetrators will continue to develop ingenious ways to circumvent regulatory systems. Because of this, relentless effort should continue to be directed toward research and development in low cost, fast and efficient means of detecting counterfeit drugs at their points of origin, in transit or on delivery, to give the regulatory bodies an edge in this fight against the heinous profession of ‘selling death to humanity’.

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REFERENCES

Acierno R, Carata E, De Pascali SA, Fanizzi FP, Maffia M, Mainetti L and Patrono L. Potential Effects of RFID systems on biotechnology insulin preparation: a study using HPLC and NMR spectroscopy. The IEEE/ICME International Conference on Complex Medical Engineering. 2010 (July 13-15):198-203.

Agarwal UP and Atalla RH (1995). Raman Spectroscopy. In: Conners, Terrance E.; Banerjee, Sukit, (Eds). Surface analysis of paper (pp. 152-181). Boca Raton, FL: CRC Press, Inc.

Alban S, Lühn S. and Schiemann S. Combination of a two-step fluorescence assay and a two-step anti-Factor Xa assay for detection of heparin falsifications and protein in heparins. Anal Bioanal Chem. 2011; 399:681–690.

Aydogmus Z. Simple and Sensitive Spectrofluorimetric Method for the Determination of Oseltamivir Phosphate in Capsules Through Derivatization with Fluorescamine. J Fluoresc. 2009, 19: 673–679

Bate R and Hess K. Anti-malarial drug quality in Lagos and Accra - a comparison of various quality assessments. Mal J. 2010; 9: 157-163.

Berman B. Strategies to detect and reduce counterfeiting activity. Bus Horizons. 2008; 51: 191-199.

Blanco M and Villarroya I. NIR spectroscopy: a rapid-response analytical tool.Trends Anal Chem. 2002; 21: 240-250.

Boiret M, Meunier L and Ginot Y-M. Tablet potency of Tianeptine in coated tablets by near infrared spectroscopy: Model optimisation, calibration transfer and confidence intervals. J Pharmaceut Biomed. 2011; 54: 510-516.

Bugay DE and Brush RC. Chemical Identity Testing by Remote-Based Dispersive Raman Spectroscopy. Appl Spectrosc. 2010; 64: 467-475.

Burki T. The real cost of counterfeit medicines. Lancet Infect Dis. 2010; 10: 585-586.

Cahyadi C, Karande AD, Chan LW, Heng PWS. Comparative study of nondestructive methods to quantify thickness of tablet coatings Int J Pharm. 2010, 398: 39-49.

Casola L, Kemp S and Mackenzie A. Consumer decisions in the black market for stolen or counterfeit goods. J. Economic Psychology. 2009; 30:162-171.

Caudron J-M, Ford N, Henkens M, Mace C, Kiddle-Monroe R and Pinel J. Substandard medicines in resource-poor settings: a problem that can no longer be ignored. Trop Med Inter health. 2008; 13: 1062-1072.

Costa AM. The economics of crime: A discipline to be invented and a Nobel Prize to be awarded. J. Policy Model. 2010; 32:648-661.

Davey EA, Zucchero AJ, Trapp O and Bunz UHF. Discrimination of Organic Acids Using a Three Molecule Array Based upon Cruciform Fluorophores. J Am Chem Soc. 2011; 133: 7716–7718.

De C Marquesa FF, da Cunha ALMC, Aucélio RQ. Laser induced fluorescence and photochemical derivatization for trace determination of camptothecin.Talanta. 2010; 83: 256-261.

De Peinder P, Vredenbregt MJ, Visser T, de Kaste D. Detection of Lipitor® counterfeits: A comparison of NIR and Raman spectroscopy in combination with chemometrics. J Pharmaceut Biomed. 2008; 47: 688–694.

Ding M, Wu X, Yuan L, Wang S, Li Y, Wang R, Wen T, Du S, and Zhou X. Synthesis of core–shell magnetic molecularly imprinted polymers and detection of sildenafil and vardenafil in herbal dietary supplements. J. Hazard Mater. 2011; 191: 177–183.

Domike R, Ngai S and Cooney CL. Light induced fluorescence for predicting API content in tablets: Sampling and error. Int J Pharm. 2010; 391: 13–20.

Dondorp AM, Yeung S, White L, Nguon C, Day NPJ, Socheat D and von Seidlein L. Artemisinin resistance: current status and scenarios for containment. Nat Rev Microbiol. 2010; 8: 272-280.

Dong Y-D and Boyd BJ. Applications of X-ray scattering in pharmaceutical science. Int J Pharm. 2011 in press.

Dowell FE, Maghirang EB, Fernandez FM, Newton PN and Green MDJ. Detecting counterfeit antimalarial tablets by near-infrared spectroscopy. Pharm Biomed Analysis. 2008; 48: 1011-1014.

Eliasson C, Macleod NA, Jayes LC, Clarke FC Hammond SV, Smith MR and Matousek P. Non-invasive quantitative assessment of the content of pharmaceutical capsules using transmission Raman spectroscopy. J Pharmaceut Biomed. 2008; 47: 221–229.

Eliasson C and Matousek P. Noninvasive Authentication of Pharmaceutical Products through Packaging Using Spatially Offset Raman Spectroscopy. Anal Chem. 2007; 79: 1696-1701.

Golightly RS, Doering WE and Natan MJ. Surface-Enhanced Raman Spectroscopy and Homeland Security: A Perfect Match? ACSNano. 2009; 3: 2859–2869.

Hollis DP. Quantitative Analysis of Aspirin, Phenacetin, and Caffeine Mixtures by Nu clear Magnetic Resonance Spectrometry. Anal Chem. 1963; 35: 1682-1684.

Holzgrabe U. Quantitative NMR spectroscopy in pharmaceutical applications. Prog Nucl Mag Res Sp. 2010; 57: 229-240.

Holzgrabe U and Malet-Martino M. Analytical challenges in drug counterfeiting and falsification—The NMR approach. J Pharmaceut Biomed. 2011; 55: 679–687.

Izake EL. Forensic and homeland security applications of modern portable Raman spectroscopy. Forensic Sci Int. 2010; 202: 1-8.

Kauppila TJ, Flink A, Haapala M, Laakkonen U-M, Aalberg L, Ketola RA and Kostiainen R. Desorption atmospheric pressure photoionization–mass spectrometry in routine analysis of confiscated drugs. Forensic Sci Int. 2011; 210: 206–212.

Kwok SK, Ng OPH,Tsang AHC and Liem HM. Physimetric identification (Physi-ID)—Applying biometric concept in physical object identification Computers in Industry. 2011; 62: 32-41.

Kwok SK, Ting JSL, Tsang AHC, Lee WB and Cheung BCF. Design and development of a mobile EPC-RFID-based self-validation system (MESS) for product authentication. Comput Ind. 2010; 61: 624–635.

Lai CK, Zahari A, Miller B, Katstra WE, Cima MJ and Cooney CL. Nondestructive and On-line Monitoring of Tablets Using Light-Induced Fluorescence Technology. AAPS Pharm Sci Tech. 2004; 5: 1-10.

Lakafosis V, Traille A, Lee H, Gebara E, Tentzeris MM, DeJean G and Kirovski D. RFID-CoA: The RFID tags as Certificates of Authenticity. IEEE International Conference on RFID. 2011; 207-214.

Lal R, Ramachandran S and Arnsdorf MF. Multidimensional Atomic Force Microscopy: A Versatile Novel Technology for Nanopharmacology Research. The AAPS J. 2010; 12: 716-728.

Lee H-M, Kim CS, Jang YM, Kwon SW and Lee B-J. Separation and structural elucidation of a novel analogue of vardenafil included as an adulterant in a dietary supplement by liquid chromatography–electrospray ionization mass spectrometry, infrared spectroscopy and nuclear magnetic resonance spectroscopy. J Pharmaceut Biomed. 2011; 54: 491-496.

Lehtonen M, Ostojic D, Ilic A and Michahelles F. Securing RFID Systems by Detecting Tag Cloning. LNCS. 2009; 5538: 291-308.

Likar MD, Cheng G, Mahajan N and Zhang Z. Rapid identification and absence of drug tests for AG-013736 in 1mg Axitinib tablets by ion mobility spectrometry and DARTTM mass spectrometry. J Pharmaceut Biomed. 2011; 55: 569–573.

Lin W-Q, Jiang J-H, Yang H-F, Ozaki Y, Shen G-L and Yu R-Q. Characterization of Chloramphenicol Palmitate Drug Polymorphs by Raman Mapping with Multivariate Image Segmentation Using a Spatial Directed Agglomeration Clustering Method. Anal Chem 2006; 78: 6003-6011.

Lopes MB, Wolff J-C, Bucas-Dias JM and Figueiredo MAT. Determination of the composition of counterfeit HeptodinTM tablets by near infrared chemical imaging and classical least squares estimation. Analytica Chimica Acta. 2009; 641: 46–51.

Maia PMS, da Cunha ALMC, de C Marques FF and Aucelio RQ. Room-temperature phosphorimetry for the determination of trace contaminations of camptothecin in anticancer drugs Microchem J. 2010; 96: 108-113.

Marini RD, Rozet E, Montes MLA, Rohrbasser C, Roht S, Rhème D, Bonnabry P, Schappler J, Veuthey J-L, Hubert Ph and Rudaz S. J. Reliable low-cost capillary electrophoresis device for drug quality control and counterfeit medicines. Pharmaceut Biomed. 2010; 53: 1278-1287.

Mazel V, Reiche I, Busignies V, Walter P, Tchoreloff P. Confocal micro-X-ray fluorescence analysis as a new tool for the non-destructive study of the elemental distributions in pharmaceutical tablets. Talanta. 2011; 85: 556–561.

Morzunova TG. Capillary Electrophoresis in Pharmaceutical Analysis (A Review). Pharm Chem J-USSR. 2006; 40: 158-170.

Newton PN, Green MD, Fernandez FM, Day NPJ and White NJ. Counterfeit anti-infective drugs. Lancet Infect Dis. 2006; 6: 602-613.

Newton PN, Green MD and Fernandez FM. Impact of poor-quality medicines in the 'developing' world. Trends Pharmacol Sci. 2010; 31: 99-101.

Novoa-Carballal R, Fernandez-Megia E, Jimenez C and Riguera R. NMR methods for unravelling the spectra of complex mixtures. Nat Prod Rep. 2011; 28: 78-98.

Pang W, Yang H,Wu Z, Huang M and Hu J. LC-MS–MS in MRM Mode for Detection and Structural Identification of Synthetic Hypoglycemic Drugs Added Illegally to 'Natural' Anti-Diabetic Herbal Products. Chromatographia. 2009; 70: 1353-1359.

Puchert T, Lochmann D, Menezes JC and Reich G. Near-infrared chemical imaging (NIR-CI) for counterfeit drug identification—A four-stage concept with a novel approach of data processing (Linear Image Signature). J. Pharmaceut Biomed. 2010; 51: 138–145.

Puchert T, Lochmann D, Menezes JC and Reich G. A multivariate approach for the statistical evaluation of near-infrared

chemical images using Symmetry Parameter Image Analysis (SPIA). Eur J Pharm Biopharm. 2011; 78: 117–124.

Ricci C, Nyadong L, Fernandez FM, Newton PN and Kazarian SG. Combined Fourier-transform infrared imaging and desorption electrospray-ionization linear ion-trap mass spectrometry for analysis of counterfeit antimalarial tablets. Anal Bioanal Chem. 2007; 387:551–559.

Rodionova O, Pomerantsev A, Houmøller L, Shpak A and Shpigun O. Noninvasive detection of counterfeited ampoules of dexamethasone using NIR with confirmation by HPLC-DAD-MS and CE-UV methods. Anal Bioanal Chem. 2010; 397: 1927-1935.

Rodionova OY and Pomerantsev AL. NIR-based approach to counterfeit-drug detection. Trends Anal Chem. 2010; 29: 795-803.

Roggo Y, Chalus P, Maurer L, Lema-Martinez C, Edmond A and Jent N. A review of near infrared spectroscopy and chemometrics in pharmaceutical technologies. J. Pharmaceut Biomed. 2007; 44: 683–700.

Silvestre V, Mboula VM, Jouitteau C, Akoka S, Robins RJ, Remaud GS. Isotopic ^{13}C NMR spectrometry to assess counterfeiting of active pharmaceutical ingredients: Site-specific 13C content of aspirin and paracetamol. J Pharmaceut Biomed. 2009; 50: 336–341.

Staub A, Rudaz S, Veuthey J-L, Schappler J. Multiple injection technique for the determination and quantitation of insulin formulations by capillary electrophoresis and time-of-flight mass spectrometry. J Chromatogr A. 2010; 1217: 8041-8047.

Thibaudeau C, Remaud G, Silvestre V and Akoka S. Performance Evaluation of Quantitative Adiabatic ^{13}C NMR Pulse Sequences for Site-Specific Isotopic Measurements. Anal Chem. 2010; 82: 5582–5590.

Vajna B, Farkas I, Szabó A, Zsigmond Z, Marosi G. Raman microscopic evaluation of technology dependent structural differences in tablets containing imipramine model drug. J. Pharmaceut Biomed. 2010; 51: 30-38.

Valeur B. From Well-Known to Underrated Applications of Fluorescence. Ser Fluoresc. 2008; 4: 21–43.

Venhuis BJ, Zomer G, Vredenbregt MJ and de Kaste D. The identification of (−)-trans-tadalafil, tadalafil, and sildenafil in counterfeit Cialis® and the optical purity of tadalafil stereoisomers. J. Pharmaceut Biomed. 2010; 51:723–727.

Venhuis BJ, Vredenbregt MV, Kaun N, Maurin JK, Fijałek Z and de Kaste D. The identification of rimonabant polymorphs, sibutramine and analogues of both in counterfeit Acomplia bought on the internet. J. Pharmaceut Biomed. 2011; 54: 21–26.

Vredenbregt MJ, Blok-Tip L, Hoogerbrugge R, Barends DM and de Kaste D. Screening suspected counterfeit Viagra® and imitations of Viagra® with near-infrared spectroscopy. J. Pharmaceut Biomed. 2006; 40: 840–849.

Wawer I, Pisklak M and Chilmonczyk Z. ^{1}H, ^{13}C, ^{15}N NMR analysis of sildenafil base and citrate (Viagra) in solution, solid state and pharmaceutical dosage forms. Pharmaceut Biomed. 2005; 38: 865–870.