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Short communication

Counterfeit drugs detection by measurement of tablets and secondary packaging colour

Andrea Luca Rodomonte[a,*], Maria Cristina Gaudiano[a], Eleonora Antoniella[a], Daniela Lucente[b], Virginia Crusco[b], Monica Bartolomei[a], Paola Bertocchi[a], Livia Manna[a], Luisa Valvo[a], Franco Alhaique[b], Nadia Muleri[b]

[a] Dipartimento del Farmaco, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy
[b] Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

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ABSTRACT

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. This paper intends to evaluate the possibility to employ for preliminary examinations of suspicious samples an optical spectrophotometer (colorimeter) used in the graphic industry, capable of measuring the reflectance visible spectrum of solid materials. The colorimeter was tested on original and counterfeited Viagra, Cialis and Levitra by measuring the colour of tablets' surface and of a specific spot of the packages. Various batches of the original drugs were employed both to investigate precision and robustness of the technique and to build spectral libraries. These libraries were used to compare suspicious samples to the corresponding original by means of a wavelength distance pattern recognition method. The method was eventually tested on suspicious samples sized by police authorities in order to evaluate its effectiveness. The device resulted precise and robust toward ambient conditions changes, although some limits emerged: the libraries of original samples need a frequent update and a lower precision is to be expected for tablets which surface is extremely convex.

1. Introduction

The pharmaceutical counterfeiting phenomenon is a major public health risk [1] which is being intensively debated these days [2]. To respond properly to the alarming raise of the phenomenon, WHO has created a global coalition of stakeholders and regulatory agencies called IMPACT (International Medical Products Anti-Counterfeiting Taskforce) [3] whose goal is mainly to forge international collaboration and raise awareness of the dangers posed by counterfeit medicines consumption.

Many different methodologies to detect counterfeiting by various analytical techniques have been reported to date [4–19]. However the raising concern about counterfeiting 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. Moreover such methods would be of great advantage to developing countries where more effective testing procedures are often lacking. To this purpose in recent years colorimetry and refractometry on drug solution have been proposed with success [20–24] also in conjunction [25].

This paper intends to present another possible field method not yet reported: the detection of counterfeiting by measurement of tablet and packaging colour. This study investigated the effectiveness and reliability for in the field inspections of an optical spectrophotometer (colorimeter) capable of measuring the reflectance visible spectrum of solid materials. This particular instrument, which is generally used in the graphic industry, basically projects a light toward a solid surface, collects the reflected spectrum and digitally records it. Therefore in principle one can match the spectrum from the surface of a suspicious drug with the one of the corresponding original and, once the right statistical treatment of data is put in place, establish with a defined amount of confidence if the sample is genuine, fake, or deserves further investigations.

As a model three of the most counterfeited drugs were considered: Viagra® from Pfizer, Cialis® from Lilly and Levitra® from

* Corresponding author. Tel.: +39 0649902627; fax: +39 649903854.
E-mail address: andrea.rodomonte@iss.it (A.L. Rodomonte).

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Bayer. Colours of both tablet's surface and of secondary package (i.e. the carton box) were considered. An area of the package where the colour was more distinctive was chosen (i.e. the green side in the Cialis box, the violet in the Levitra and the blue in the Viagra).

2. Experimentals

2.1. Chemicals

Original Viagra®, Cialis® and Levitra® were mostly bought from Italian pharmacies; about a 10% of the samples were bought from online UK pharmacies in order to widen the variability among batches. More than one dosage for each drug was considered (i.e. 25, 50 and 100 mg for Viagra, 10 and 20 mg for Cialis and 10 and 20 mg for Levitra) and tested separately, in order to verify the influence of tablet dimension on the colorimeter performance. Also secondary packages were tested separately for each dosage because they might have come from different production lines. Ten to twenty batches were analysed for each dosage. Batches production date spanning 5 years were chosen to maximise heterogeneity. Tablets were scanned on the less carved side (which for Viagra and Levitra is the one indicating the brand, while for Cialis is the one not carved at all) to avoid introducing a source of heterogeneity.

For those tablets of irregular shape the operators were instructed to make the scan always on the same spot.

2.2. The colorimeter

The colorimeter employed was the *eye-one* model by *X-rite* (Regensdorf, Switzerland). A first typical entry-level use of this kind of colorimeter is to calibrate pc monitors and video-projectors to get the accurate set of colours even on a different monitor from that on which the image was created. A second typical use is the monitor-to-printer matching, a process that ensures photographers and designers that the colour they are seeing on the PC monitor display will be the same on their printed output.

This instrument is indeed portable: It is a very small object and it can be connected to every personal computer through a standard USB port, through which it is powered without the need for a dedicated battery.

The Gretag Macbeth *i1Match* monitor profiling software was employed. It permitted to have a description of each scanned colour in terms of many different scales. Most importantly it permitted to export data in Microsoft Excel format as *reflectance vs wavelength*. This data format was employed during this study in every calculation concerned.

The colorimeter covers the whole visible spectrum, from 380 to 730 nm in 10 nm steps (i.e. it scans 36 points). In Fig. 1 spectra from Cialis 20 mg, Viagra 100 mg and Levitra 10 mg tablet surface are depicted.

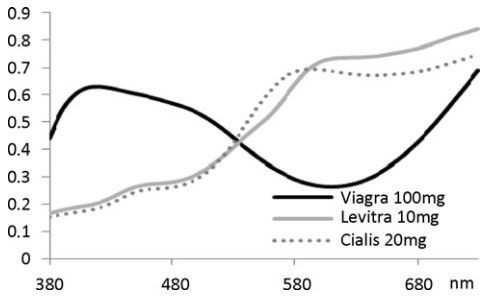

Fig. 1. Reflectance spectra obtained from tablets of Viagra, Levitra and Cialis.

Spectrum acquisition is performed placing the ocular upon the surface to be scanned, making it adhere perfectly in order to avoid interferences from ambient light (see Fig. 2).

The scanning requires not more than a couple of seconds.

Since the response to ambient light conditions was unknown prior to this study, all the analyses were performed in homogeneous conditions putting the scanner under a *GTI Mini Matcher* (GTI Graphic Technology, Inc., 211 Dupont Avenue, Newburgh, NY – www.gtilite.com) which is a booth that provides a calibrated and reproducible type of lighting (daylight, office, incandescent illumination or ultra-light).

Every 10 scans a calibration of the colorimeter was performed on a white barium sulphate tile.

2.3. Validation

2.3.1. Precision

To gain a better knowledge of method performances a validation was deemed necessary. In particular precision and ruggedness studies were devised.

The precision study was designed with the purpose of estimating method imprecision and comparing it with the batch to batch variability. It was also designed in order to try to evaluate which step of the analysis contributed most to the overall imprecision.

In order to do this a three-factor nested ANOVA experiment was conducted, the three factors being operators, days and replicates. Specifically, for every dosage of each drug considered three operators made 10 replicates in two different days on each batch available. Then a precision value was calculated for each dosage of the three drugs.

This procedure permitted to know for each batch separately both the total method precision and its various components (namely inter-operator precision, inter-day precision and repeatability). The pooling of the total precisions of each batch provided

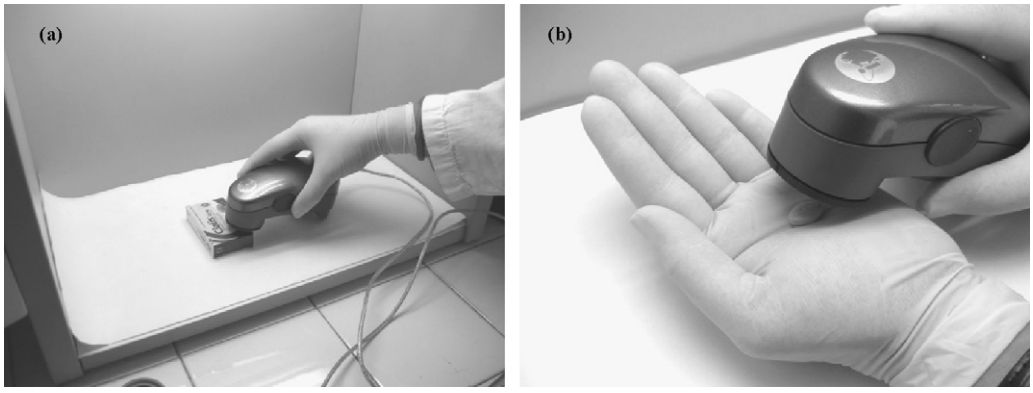

Fig. 2. (a) The scanning of Cialis 20 mg secondary packaging. (b) The scanning of a tablet.

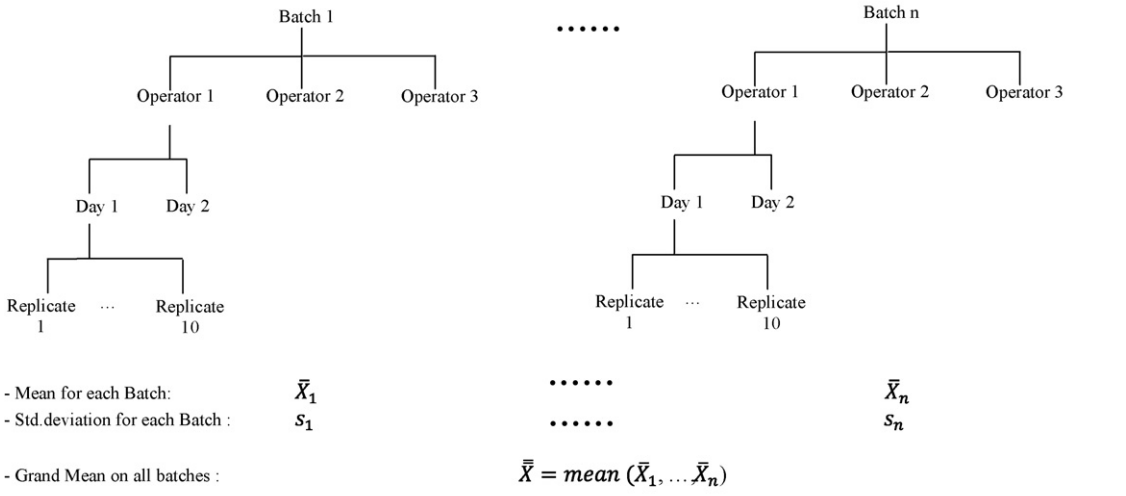

Fig. 3. A scheme of the experimental design is reported together with the statistical data collected.

then the total method precision for the specific dosage. See Fig. 3 for a schematic view of the experimental design for each drug and dosage.

Since the colours obtained in all the 10 replicates were displayed on the monitor at the same time, the operator could immediately evaluate if he had committed any blunder (ambient light can ruin the acquisition if the ocular is not perfectly in place) and replace it with a new one.

Both Cochran and Grubbs tests were conducted following indication from [26–28].

Apart from the nested one also a crossed layout of data was designed to carry out a one-way ANOVA: this in order to evaluate whether the method imprecision was significant compared to the batch to batch heterogeneity. In particular a matrix was built of n columns (one for each batch) by six rows (the data collected in 2 days by three operators; the 10 replicates made each day by every operator were averaged to simplify calculations). See Table 1. To evaluate if batch to batch variability was significant in comparison to method imprecision an F test was then carried out: the ratio between the batch to batch *mean square* and the residual *mean square* was calculated and compared to the tabulated F for k-1 and 6·k–k degrees of freedom (see for example [29] for a more detailed discussion about the significance of variance sources in ANOVA).

2.3.2. Ruggedness

Ruggedness studies were conducted on both packaging and tablets of all the three drugs at every dosage. The parameters deemed interesting were ambient light, temperature and sampling. Each one was investigated at two levels as in the Plackett–Burman experimental design [30]. Analyses were carried out respectively: in a dark room or with all lights turned on under the *Mini Matcher* booth described in Section 2.2; at 20 °C and at 30 °C; laying the tablet on a plane surface or holding it in the palm of the hand (this of course was not tested for packaging).

2.4. Analyses of suspicious samples

After completing validation the method was applied to suspicious samples bought from online pharmacies or collected from seizures by the Italian police forces.

To evaluate if a suspicious sample was genuine or counterfeit a simple *wavelength distance pattern recognition* method was employed as it's generally done for other spectroscopic techniques (e.g. near infrared spectroscopy). All the spectra from precision studies were considered part of the training set in this work. Then a grand mean $\bar{x}_i$ and a standard deviation s_i at each wavelength i were calculated on all the spectra collected. The grand mean was the one from Fig. 3 and the standard deviation was the total standard deviation from all the measurements collected during the training stage. This total standard deviation of course comprised both method imprecision and batch to batch heterogeneity (see Fig. 3).

To test the suspicious medicines their residual spectrum made of 36 normalized deviation Z_i (one for each wavelength in the spectrum collected by the colorimeter) was calculated according to

Table 1
One-way crossed ANOVA layout used to evaluate significance of method imprecision in comparison to batch-to-batch heterogeneity.

	Batch 1	Batch 2	...Batch j...	Batch k
	$X_{operator1\ day1\ batch1}$	$X_{operator1\ day1\ batch2}$	$X_{operator1\ day1\ batch\ j}$	$X_{operator1\ day1\ batch\ k}$
	$X_{operator1\ day2\ batch1}$	$X_{operator1\ day2\ batch2}$	$X_{operator1\ day2\ batch\ j}$	$X_{operator1\ day2\ batch\ k}$
	$X_{operator2\ day1\ batch1}$	$X_{operator2\ day1\ batch2}$	$X_{operator2\ day1\ batch\ j}$	$X_{operator2\ day1\ batch\ k}$
	$X_{operator2\ day2\ batch1}$	$X_{operator2\ day2\ batch2}$	$X_{operator2\ day2\ batch\ j}$	$X_{operator2\ day2\ batch\ k}$
	$X_{operator3\ day1\ batch1}$	$X_{operator3\ day1\ batch2}$	$X_{operator3\ day1\ batch\ j}$	$X_{operator3\ day1\ batch\ k}$
	$X_{operator3\ day2\ batch1}$	$X_{operator3\ day2\ batch2}$	$X_{operator3\ day2\ batch\ j}$	$X_{operator3\ day2\ batch\ k}$
Mean	$\bar{X}_1$	$\bar{X}_2$	$\bar{X}_j$	$\bar{X}_k$
Variance	s_1^2	s_2^2	s_j^2	s_k^2
Grand mean	$\bar{\bar{X}}$			

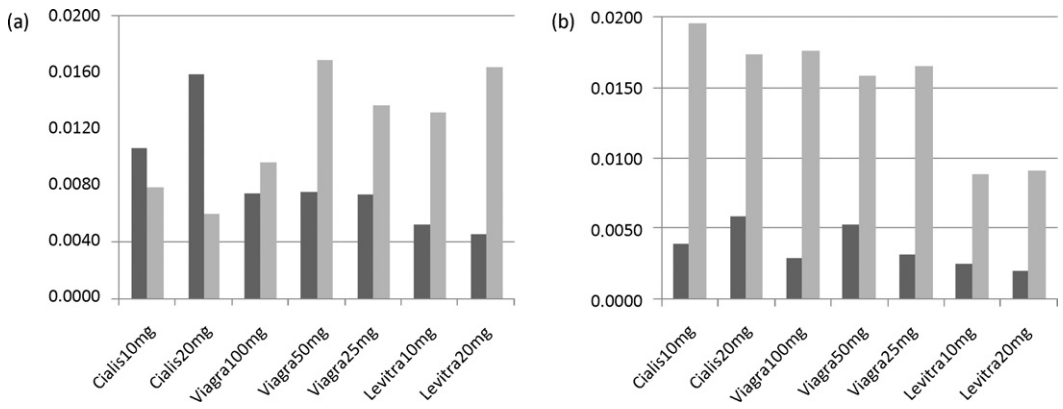

Fig. 4. The maximum absolute values of standard deviation components from ANOVA experiments due to batch-to-batch heterogeneity (in light grey) and method imprecision (dark grey) are reported. In (a) tablet values are reported, in (b) packaging values are reported. Only the wavelength ranges of the specific colour were considered, i.e. for tablets: 560–620 nm for Cialis (yellow–orange), 400–490 nm for Viagra (azure), 560–620 nm for Levitra (yellow–orange); for packages: 490–560 nm for Cialis (green), 400–510 nm for Viagra (blue), 400–530 for Levitra (violet).

equation:

$$Z_i = \frac{x_i - \bar{x}_i}{s_i}$$

where $\bar{x}_i$ is the grand mean at each wavelength i from the training set of genuine samples, x_i is the reflectance of the suspicious sample at each wavelength i and s_i the standard deviation of the training set at each wavelength i.

A sample was considered genuine when all its Z_i were $\leq$ of the tabulated Z_{crit}, the critical value of Z from the two-sided Student's distribution at 95% confidence level (i.e. 1.96)[1]; the opportunity to carry on further analyses with classical techniques is then to be evaluated on a case to case basis.

When one or more Z_i resulted $> Z_{crit}$ two cases were distinguished based on the Z_i failing the test being or not related to wavelengths in the range of the specific colour of the sample (for example this range is 560–620 nm for the yellow–orange colour in Cialis and Levitra, or 490–560 nm for the green in the carton box of Cialis, etc.): when the $Z_i > Z_{crit}$ fell in the range of the specific colour, the sample was considered a counterfeit; when the $Z_i > Z_{crit}$ fell outside this range, the sample was considered dubious. This distinction had the sole purpose of better studying the colorimeter performances: for future in-the-field uses of the colorimeter inspectors should be instructed to bring the sample to the laboratory in any case.

3. Results and discussion

3.1. Validation

3.1.1. Precision studies

Based on the data shown in Fig. 4, the following evidences were collected:

- Batch to batch heterogeneity accounted for almost the entire variability of the *training set* for all the packages and most of the tablets. This was not true for those tablets showing a markedly convex shape: Cialis 20 mg and 10 mg and in a less extent Viagra 100 mg. In these specific cases the method imprecision (which is the combination of inter replica, inter-day and inter-operator variability) was greater or comparable (in the case of Viagra) to batch to batch heterogeneity. This means that the colorimeter is sufficiently precise when the surface to be analysed is flat (as in packages) or not very convex (as in many tablets), but its precision diminish considerably with the convexity of the sample surface.
- In Cialis (and in a less extent in Viagra) batch to batch variability proved slightly larger for packages then for tablets. This shows that the physico-chemical process of tablet filming is more controlled and reproducible then the process of printing on the cartons; the mixture of colouring agents used for the tablet filming is standardized with a higher degree of accuracy in comparison to boxes colour, which is instead apparently more prone to variation from one lot to another.
- The nested ANOVA experiment showed that the three factors investigated contribute to a different extent to the global precision of the method (not reported in Fig. 4): the replicates contribution (the short term repeatability of a single operator) is less significant than that from either operator or day. Operator and day variability are similar, although the first one is smaller for packages. The reason for this resides in the role of the operator being minimal in the scanning of boxes, because their surface is flat. On the contrary a more pronounced operator to operator variability was noticed for tablets, because in this case the operator ability in handling the tablet played a major role. As expected this resulted particularly true for Cialis 20 mg, because of its marked convexity.

3.1.2. Ruggedness studies

Ruggedness experiments confirmed what was hinted by precision experiment about tablets handling. The only parameter that may have a perceptible influence on the final result is in fact the way in which the sample is handled, especially for Cialis tablets. This confirms the importance of a proper training of the operator: to obtain a reliable result he has to let the scanner adhere as perfectly as possible to the tablet surface in order to avoid ambient light to interfere with the analysis and most importantly he has to hold the tablet firmly in order to avoid wobbling, which could caused a certain amount of variability among repetitions.

Temperature and ambient light, instead, did not show any influence on the analysis at least in the investigated range.

[1] In wavelength distance pattern recognition methods generally larger tolerances are allowed compared to the 95% chosen in this study: often Z_{crit} up to a value of 6 are used, depending on the number of trials in the set and of wavelengths considered [31]. Anyway it should be noted that these methods are normally used in quality control to ascertain if a certain sample fulfils the quality standard set by the manufacturer, so the risk in such a case is that of erroneously discarding a good sample. In the specific case of counterfeiting detection, instead, the major danger lies in a bad sample being labelled as genuine. Thus the need for a more cautious choice of Z_{crit} (see [31] for a detailed description of this matter).

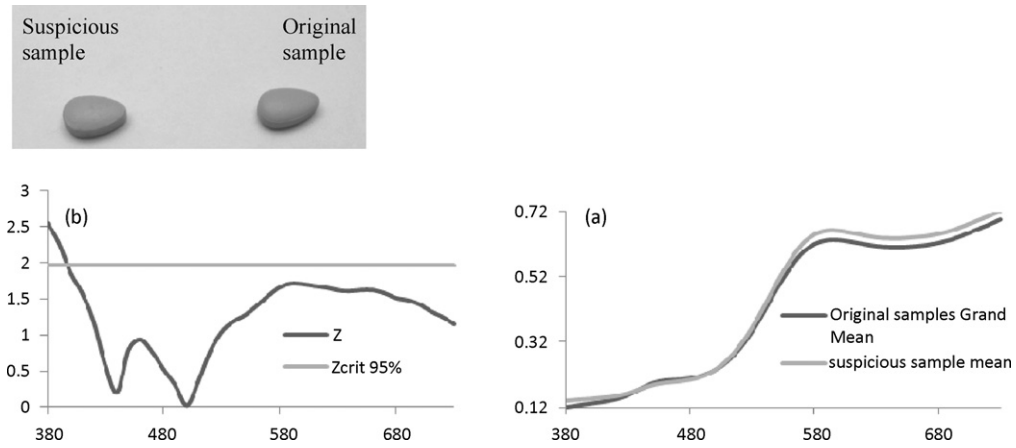

Fig. 5. An original 20 mg Cialis tablet and a tablet indistinguishable from it are presented together with their spectra (a) and the *Z* test results (b) for every wavelength. It can be seen that there are only a few *Z* values trespassing the Z_{crit} cut-off, and they lies outside the 560–620 nm range, which is the range specific of the yellow/orange colour of Cialis. So the sample still failed to pass the test but was classified as deserving further investigations by other techniques (see classification described in Section 2.4).

3.2. Analysis of suspicious samples

Suspicious samples were collected from police seizures or purchased from online pharmacies.

Most of them did not have any secondary package so only the results obtained with tablets will be discussed here. A total of 38 samples were collected containing 2–30 tablets each. Approximately a 40% was constituted by Viagra, another 40% by Cialis and 20% by Levitra. All the samples underwent a preliminary visual inspection by an attentive comparison with the originals: Eleven were sorted as quite different in colour from the corresponding original, seventeen as faintly different and thirteen as extremely similar or indistinguishable. All the samples were analysed with the method proposed (see Section 2.4). After the colorimetric analysis they were analysed with HPLC/UV and IR spectroscopy for confirmation: all the samples resulted counterfeited apart from 6 samples in the "extremely similar" group, which were in fact genuine.

The colorimeter correctly classified as counterfeit with no need for further investigations all the tablets from group one and two. Of the thirteen tablets in group 3 the colorimeter correctly identified all the samples apart from one extremely good looking Cialis fake tablet which was classified as dubious and deserving further analysis according to the approach in Section 2.4. This sample and a rather good looking fake Viagra of group 3 are reported respectively in Figs. 5 and 6.

Although these results are apparently quite good, it should be stressed again that while method validation proved that the colorimeter is sufficiently precise for most kind of tablets and packages, in the case of Cialis the moderate imprecision due to its peculiar convexity may lead to wrong responses when extremely accurate counterfeits are analyzed. At the moment the small number of extremely accurate Cialis counterfeits tested does not allow for conclusive statements on this issue.

Some tests were also made to check the ability of the method to correctly identify genuine materials. A variety of genuine samples were tested. At the probability level chosen about a 25% of packages and a 15% of tablets were wrongly identified as counterfeit. Moreover some packages spectra from Viagra original samples bought

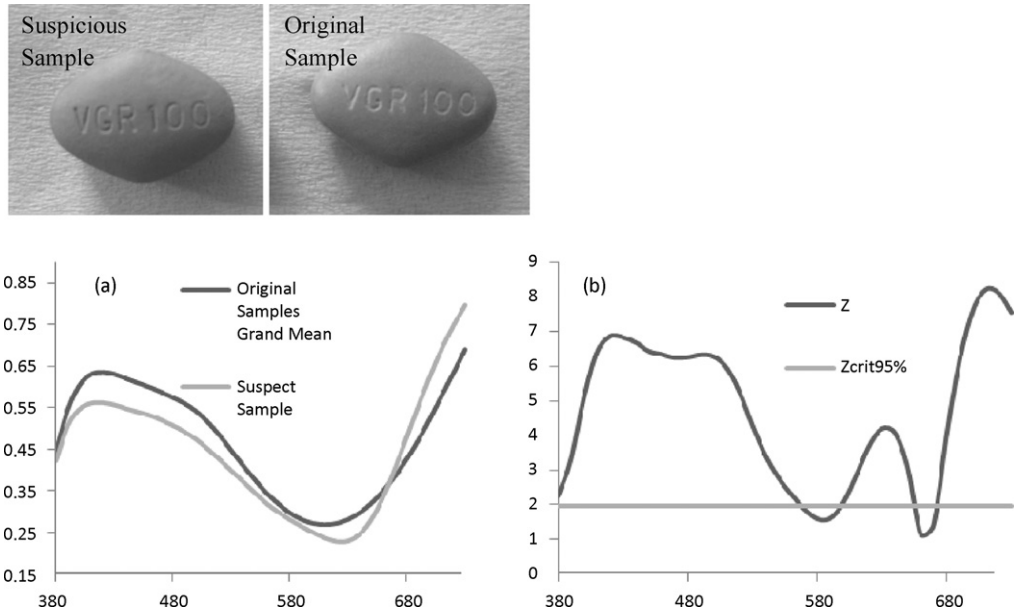

Fig. 6. An original 100 mg Viagra tablet and a tablet almost indistinguishable from it are presented together with their spectra (a) and the *Z* test results (b) for every wavelength. It can be seen that the calculated *Z* value is much greater than the critical one for most of the wavelengths so the tablet failed to pass the test and was correctly identified as counterfeit.

in this second stage resulted quite different from the library spectra. If one excludes the possibility to have incurred in counterfeits (which is indeed quite unlikely having bought the samples from a regular pharmacy) the only explanation for this is that Viagra packages colour is not as much reproducible as the tablets one is. This seemingly proves that for Viagra package (but it may also be true for other packages not tested here) there is a frequent change in colour from the manufacturer that may compromise the possibility to use effectively the colorimeter. Levitra and Cialis package colour resulted instead more reproducible. However anyone wanting to apply the proposed technique should absolutely take care to constantly update the spectral library especially for packages. Moreover package testing may result somehow more tricky then tablets because European market allows foreign packaging for each EU country. Even if for the erectogenic drugs employed here as model no significant differences were observed for packages coming from different countries, this may be an issue for other products not tested in this study. So in principle a database should better be made of samples from as many countries as possible. Also stickers that cannot be removed may be present on the package place assigned for the measurement. In this case of course the inspector has to check the sole tablet.

4. Conclusions

The investigated technique is very fast, inexpensive and portable. Thus in the future its use in conjunction with a laptop pc can be envisaged as a mean for instant screening by police or custom authorities prior to bringing the samples to any official control laboratory. Moreover it can be used without any personnel high level analytical training and could be of benefit when more effective testing procedures are lacking.

This technique may indeed help in the very first investigation of suspected counterfeit sample: the visual inspection. This is normally conducted by human eye which is not sufficiently accurate to detect minor differences. Moreover while a visual inspection by human eye is often prone to subjective judgments, an objective instrumental observation can assure a greater reproducibility and, most importantly, provided a good electronic data bank is available, it would spare inspectors from carrying with them the original samples for comparison.

The slow step of this technique, of course, is the building and maintaining of a library of original drugs. But once the library is in place, the whole analytical procedure is quite fast.

It also resulted sufficiently precise for packages and most of the tablets investigated and robust toward changes in ambient conditions.

However some limits emerged very clearly: first for some of the packages the production process undergoes perceptible changes during the years thus raising the chances of wrongly identifying a genuine product as counterfeit; second the colorimeter may be not sufficiently precise when tablets convexity is too pronounced.

In conclusion even if indistinguishable counterfeits are yet to be analysed in large numbers (only 11 where collected to this date, 6 of which were later proven authentic) to conclusively prove the effectiveness of the equipment, in the meantime it remains a promising choice as a support for in the field inspectors that have to decide whether to collect a sample for the laboratory or not and for those developing countries that cannot afford high tech analytical devices.

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