Recent developments of Raman spectroscopy for the qualitative analysis of falsified and substandard medicines

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Introduction

Quality medicines are essential to any health care system and are the final goal of any pharmaceutical manufacturer. Unfortunately, as stated recently by the WHO, one in ten medical products circulating in low- and middle-income countries is either substandard (out of specification products) or falsified (SF products) [1]. Although the exact prevalence of SF products is unknown in high-income countries, it remains a major risk [2] especially because of the internet market that is difficult to control [3].

The term “quality medicines” may recover different realities but, broadly speaking, one may expect the marketed medicines to be compliant with the local market specifications. These specifications ensure that the correct active ingredient (identity testing) is present in the right amount (assay testing), that the API is bioavailable (dissolution testing) and that the amount of impurities is low enough (impurity testing). Even if it is sometimes possible to identify visually SF products, only the pharmaceutical products testing following the complete pharmacopoeia monographs ensures the final quality and safety for the patient. However, this testing is long and requires a lot of high-level equipment and staff. These limitations reduce the quantity of possibly tested samples and are impossible to implement for field-testing during inspections.

Therefore, there is an increasing need for fast, reliable and, if possible, portable solutions to detect SF products. Some solutions have been proposed and are currently used on the field (e.g. GPHF Minilab™) but the number of possibly tested samples remains relatively low due to the need of consumables and the analysis time. Thanks to its non-destructive and fast character Raman, spectroscopy is more and more used in the field of SF product testing. Furthermore, it is possible to analyse samples directly through the blister (except for aluminium blister). Since both the chemical and the physical state of the sample influence Raman spectroscopy and that the intensity of the Raman scattered signal is dependent of the quantity of scattering chemical bonds, many qualitative and quantitative information could be drawn from the samples.

Results

Perhaps the first legitimate question when challenging a suspect sample is “Is the product what it is supposed to be?”. This question is however broader than what implicitly implies the former designation of SF products “counterfeit medicine”. Indeed, beside the intellectual property issues, qualitative chemical information is also very important from a public health point of view.

Raman spectrum of a finished pharmaceutical product can be seen as a unique fingerprint of the product. Indeed, the influence of both physical and chemical information makes each formulation unique. This signature may be of different quality and more or less informative depending on the equipment and the interface between the spectrophotometer and the sample. Obviously, a higher laser power and a better spectral resolution will provide more-resolved and more intense spectra enabling a finer analysis of the sample. Once obtained, the analyst must process the spectral signatures to obtain the final understandable information. This information can be of different nature: authentication of the product (typically a Pass/Fail result) or identification of the present API or combination of API.

Authentication purposes with Raman spectroscopy is often performed by comparison with a previously built (or commercially available) spectral database using correlation coefficient, hit quality index [4] or even p-value. This approach using weak statistical tools nevertheless provides contrasted results and is only able to discriminate very different products such as no API or the presence of a another API [5,6]. Small differences between samples (e.g. discrimination of several generics) requires more advanced chemometrics. Indeed, class-modelling tools such as Soft Independent Modelling of Class Analogy (SIMCA) may provide better results. SIMCA uses a collection of spectra of the target class to model its distribution and build a defined probability (e.g. 95%) confidence interval around it. Then, each new sample is projected and its distance to the modelled class is computed. If this distance is inferior to a defined threshold value, the sample is considered as belonging to the target class, otherwise, it is considered as an outlier. The main advantage of one class modelling over classification algorithms (such as support vector machine or partial least squares) is that it only models the target class enabling a more robust discrimination of new unknown spectra [7].

Beside the spectrophotometer performances, the interfacing with the samples is very important too. Indeed, if Raman signatures may be obtained directly through transparent or even opaque plastic blisters, in case of plastic bottle storage, conventional Raman spectrophotometers will inevitably return the spectral signature of the plastic bottle and possibly some spectral features of the inner product. To circumvent this issue, one may measure the spectra using spatially offset Raman spectroscopy (SORS) [8]. When performing SORS analyses, the sample is illuminated at a certain point and the spectrum measure is taken at somewhere else (with a calibrated offset). The spectrum measured with the offset is mainly constituted of the content (e.g. pharmaceutical tablets) spectral features rather than the container (e.g. plastic of the bottle) spectral features. Although the technology is not new, recently Agilent (formerly Cobalt Light) made a handheld device available on the market under the name Resolve®. Figure 1 show spectra recorded by the Resolve® device on quinine sulphate samples and paracetamol samples respectively in plastic bottle with and without SORS. As one may see, the “no SORS” spectra are highly contaminated by the plastic features of the bottle and only small spectral features can be attributed to the sample whereas, SORS spectra show relatively clean sample spectra. Nevertheless, SORS is not magic and the position of the sample in the container is crucial to obtain good quality spectra enabling further analyses. Indeed, a relatively close contact between the sample, the container and the spectrophotometer is required to obtain quality spectra.

Another limitation of Raman spectroscopy is its poor sensibility and the high interference of fluorescence. To circumvent these two issues, surface enhanced Raman spectroscopy (SERS) can be used. SERS has the property to both enhance the Raman signal and to quench fluorescence interference. Recently, several papers described the use of SERS substrates with handheld devices to detect the presence of API’s in suspect samples [9,10]. However, both approaches needed the dissolution of a fraction of the sample and its mixing with metallic colloids. Therefore, its

implementation for on-field analyses is limited due to the sample preparation and the stability of the colloidal solutions that has not yet been assessed.

Beside the global spectral signature of the sample, Raman spectroscopy may also enable the elucidation of the composition of the suspect tablets using hyperspectral imaging [11,12]. Four falsified antimalarial samples were detected using handheld devices and showed the same global signature with no trace of the expected API (artemether/lumefantrine and sulfadoxine/pyrimethamine). Hyperspectral imaging analyses were conducted and the compositions were elucidated using multivariate curve analysis (MCR-ALS). Results showed (Figure 2) the presence of contaminants (sildenafil, ciprofloxacin HCl and ciprofloxacin HCl monohydrate) at trace levels. The presence of the same contaminants in different samples enabled the grouping of the different cases and might be helpful for further forensic investigations. Raman chemical imaging might also be helpful to guide further chemical and quantitative analyses elucidating the nature of the present compounds.

Conclusions

Raman spectroscopy constitutes an essential part of the analyst's toolbox to characterize and authenticate SF drugs. Several reasons can explain this increasing interest: Raman spectra are relatively easy to interpret compared to near infrared (NIR) spectra, they may be directly compared to databases and inference is easier (e.g. identification of a wrong API). Raman spectroscopy is less sensitive to physical factors than NIR, enabling analysis of generics with a single method but is, on the other hand, less powerful as authenticating technique. Among vibrational spectroscopy techniques, Raman spectroscopy is the one for which technical developments are the highest. These developments intend to overcome the different limitations of the technique such as its poor sensitivity (e.g. SERS), fluorescence of samples (e.g. SERDS [13]) and the possibility to analyse samples through opaque containers (e.g. SORS [14]) with handheld devices opening the possibility of on-field analyses.

However, handheld devices are not designed for quantitative analyses so far. Indeed, most devices have automatic analysis time reaching a minimal signal over noise ratio. This decreases the possibility to detect substandard medicines (correct API in a lower amount). Fortunately, several advances are made in this sense (e.g. Trutools™ add-on for the Thermo Fisher's Truscan™) and portable devices have the possibility to set the analysis time and therefore accessing quantitative information.

Acknowledgment

The financial support of this research by the Walloon Region of Belgium in the framework of the Vibra4Fake project (convention n°:7517) is gratefully acknowledged.

This project has been supported by the European funds of regional development (FEDER) and by Walloon as part of the operational program "Walloon-2020.EU".

The authors would like to thank the Belgium National Fund for Scientific Research (FNRS) for the research grant offered to one of us (E. Dumont).

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Figure caption:

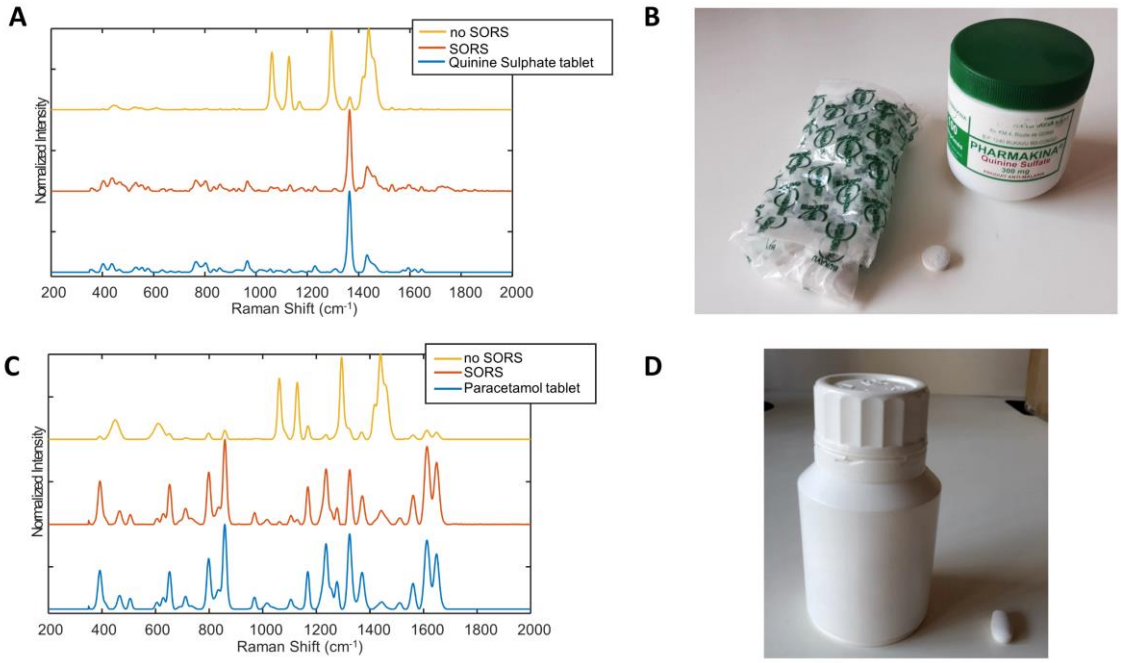

Figure 1:

A: Raman spectra of quinine sulphate tablets obtained directly on the tablet and through the container with and without SORS technology. The spectra were measured using Agilent's Resolve spectrophotometer. The correlation coefficient of the SORS and no SORS spectra with the tablet spectrum were 0.93 and 0.08 respectively.

B: Quinine sulphate tablet and container analysed.

C: Raman spectra of paracetamol tablets obtained directly on the tablet and through the container with and without SORS technology. The spectra were measured using Agilent's Resolve spectrophotometer. The correlation coefficient of the SORS and no SORS spectra with the tablet spectrum were 0.96 and 0.04 respectively.

D: Paracetamol tablet and container analysed.

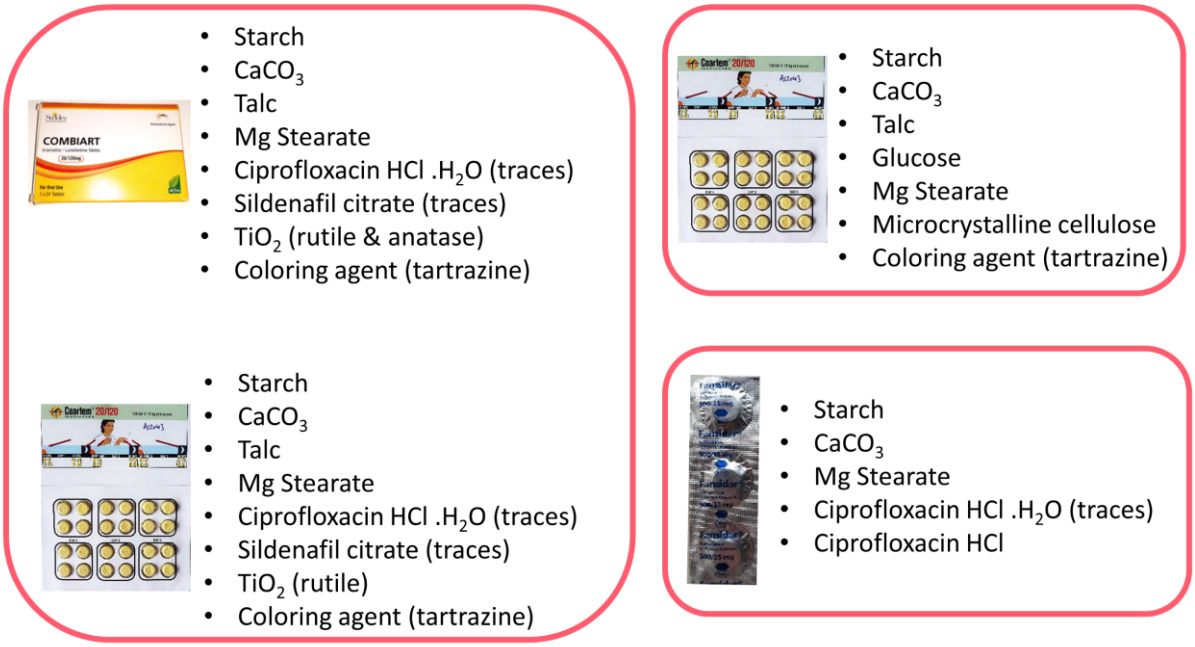

Figure 2:

Images of the packaging of the analysed samples'. The chemical composition was elucidated using Raman chemical imaging data and MCR-ALS data analysis. The two samples on the left showed similar composition and were considered as related cases whereas the two samples on the right showed different chemical composition. All four samples exhibited similar average Raman signature.