

# Contribution of Raman chemical imaging in the analysis of falsified and substandard medical products

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## Introduction

Substandard and falsified medical products are a threat to the health of patients. They are products that fail to meet quality standards or that deliberately misrepresent their identity, composition or source. They affect every region of the world and are widely available over the internet. The fight against these harmful products must be efficiently organized through prevention, communication and coordination, but also action, investigation and repression. Official laboratories for the control of medicines play an active role in the fight against falsified drugs. Analyses of suspect samples provide better knowledge of these products, identify the danger to patients and help the authorities in their decisions (Rebiere et al., 2017).

Unfortunately, substandard and falsified products are becoming more and more sophisticated and sometimes field testing or conventional analyses may not be sufficient to prevent the danger. Advanced analytical methods can be used for the exhaustive assessment of a suspect sample. Mass spectrometry and Nuclear Magnetic Resonance spectroscopy are powerful methods for this task.

Raman spectroscopy is a method that has become popular due to the highly qualitative information contained in a spectrum. Modern technologies offer access to simple portable instruments that can be used in the field, as well as research instruments coupled with microscopes with high spatial/spectral resolution.

This overview will explain the basic principle of Raman spectroscopy, and will present several applications where the method appears to be successful in the characterization of substandard and falsified medical products.

## Materials and methods

### *Samples and instrument*

The suspected samples were all obtained from seizures carried out by the French police or customs services, and the analyses were performed as per a judicial request.

Measurements were carried out on a benchtop system from Renishaw (model InVia) which is composed of a 785 nm laser diode with a nominal power of 300 mW, a Leica microscope equipped with several magnification lenses, a moving tray, a rejection filter to block the Rayleigh scattering, a diffraction grating and a CCD detector. The software (Wire) is designed to control the system, perform measurements and analyze spectra (including chemometric algorithms).

### *Raman Chemical Imaging*

Raman spectroscopy is a method that takes advantage of the vibration of molecules previously irradiated by an intense monochromatic source. As a result, two phenomena may occur: Rayleigh scattering (elastic), which is a strong signal but without any analytical interest, and Raman scattering (inelastic, i.e., with a different wavelength from that of the source), which is a signal with a very low intensity.

Conventional spectrometers use an excitation source irradiating the sample on a surface area over several mm<sup>2</sup>. When coupling with a microscope, the laser may be focused on a surface area over some μm<sup>2</sup>. This is more or less the size of a material particle in solid dosage form. Thus the Raman signal obtained may be that of one the chemical substances from the mixture.

Raman chemical imaging combines spatial and spectral information successively recording spectra at the surface of a sample at adjacent positions. The resulting data is called a hyperspectral image containing thousands of spectra gathered in a data-cube, i.e., a three-dimensional matrix with two spatial dimensions (x, y) and one spectral dimension ( $\lambda$ ). These data are generally examined in a multivariate way using chemometric algorithms such as Principal Component Analysis (PCA) or Multivariate Curve Resolution Alternating Least Square (MCR-ALS). This latter method is particularly well suited to the resolution of a spectral mixture without a-priori knowledge of the chemical system. The spectrum of a sample may be considered as the weighted sum of the spectra of pure chemical species. Thus, each pixel of the image contains the same pure signals but their contributions differ from one pixel to the next. MCR-ALS decomposes the spectral dataset into the product of the matrix of pure spectra of species and the matrix of their relative contribution in the data-cube. The use of spectral libraries helps in the identification of the resulting pure spectra.

#### *Surface Enhanced Raman Spectroscopy (SERS)*

As previously mentioned, Raman scattering is a very weak signal. This signal may be drastically enhanced with a specific sample preparation using metal nanoparticles. The use of nanoparticles suitably prepared and applied over the sample surface may enhance the Raman signal by between 100 and 10000-fold. The SERS effect is explained with two mechanisms: an electromagnetic enhancement obtained with the surface plasmon generated in the gap between two close nanoparticles, and a chemical enhancement resulting from the electron transfer between analytes and nanoparticles.

Nanoparticles are commercially available in liquid form or embedded in a substrate. In our study we decided to manufacture and characterize our own nanoparticles. A specific deposition mode was optimized for further Raman chemical imaging (Cailletaud, 2018).

## **Results and discussion**

#### *Spectroscopic screening*

Raman chemical imaging was applied on several tablets. The MCR-ALS method was used for the study of each resulting data-cube. The sample surface must preferably be flat in order to maintain the focalization of the laser. The resulting images use false colors in order to locate the different areas of the chemical pure species.

The method was applied on several kinds of samples: chloroquine tablets and anabolic tablets (Rebiere et al.,

2016). Hyperspectral images exhibit the distribution map of the compounds and identify active substances and excipients (some of them are not expected in the sample). Falsification was also established on suspect Viagra<sup>®</sup> and Plavix<sup>®</sup> samples by comparing their hyperspectral images with those of authentic ones (Rebiere et al., 2018).

#### *Detection of low dose active substances*

SERS was applied on a sample called “Anabol tablet”, previously found without any active substances using conventional Raman chemical imaging. Silver nanoparticles were prepared and applied by spray coating before performing Raman measurement over the surface of the sample. Two new pure components were detected and identified as sildenafil and ciprofloxacin. Their identification was confirmed using LC-MS and their content was found at 4  $\mu\text{g}$  sildenafil and 300  $\mu\text{g}$  ciprofloxacin per tablet. It is assumed that the presence of these two unexpected substances is due to the use of poorly cleaned manufacturing tools in which sildenafil tablets and ciprofloxacin tablets had been previously prepared by the illegal manufacturer.

## **Conclusion**

Raman chemical imaging was found to be useful for the screening of substandard and falsified samples whose composition is unknown. The identification of active substances and excipients was achieved. The method is complementary to separation methods giving the distribution map of the compounds in the samples. SERS is a sensitive method capable of detecting chemicals at a low dose content.

## **References**

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