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# RAW MATERIAL AND BULK IDENTIFICATION THROUGH RAMAN SPECTROSCOPY AND LABORATORY ANALYSIS

Relatório de Estágio do Mestrado em Engenharia Biológica e Química

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## RESUMO

Na indústria farmacêutica, as matérias-primas, antes de serem utilizadas na produção, passam por testes rigorosos para garantir que as especificações de qualidade são atingidas. Estes testes são realizados em laboratórios de Controlo de Qualidade certificados seguindo a farmacopeia europeia, o documento oficial que regula a indústria farmacêutica na Europa.

Para realizar estas análises, é necessário fazer a amostragem dos materiais na chegada à unidade. Esta etapa tem de ser realizada para cada unidade de armazenamento (SU) e para cada lote que, quando em grandes quantidades, pode atrasar a equipa do laboratório até à sua finalização. Por ser um processo moroso, desde a chegada da matéria-prima até à produção do produto, pode aumentar o risco de erros em análises quando o número de amostras a testar é elevado.

Mesmo antes da amostragem, a aplicação da técnica de espectroscopia Raman permite a redução do número de amostras e consequentemente o número de testes necessários. Isto resulta num menor tempo gasto com amostragens e análises e ainda evita contaminações por remover a necessidade de abertura da SU, pois não requer contato direto com o produto.

A aplicação desta técnica apresenta uma redução de cerca de 65% do tempo gasto no processo de amostragem e na quantidade de análises laboratoriais de um produto onde a técnica foi aplicada. Mostra assim uma melhoria significativa na produtividade, reduzindo o tempo total necessário para processar o material antes de estar pronto para uso na produção.



PALAVRAS-CHAVE: Espectroscopia Raman, Matérias-Primas, Boas Práticas de Fabrico, Controle de Qualidade, Indústria Farmacêutica.

## ABSTRACT

In the pharmaceutical industry, before being used in production, the raw materials undergo rigorous tests to ensure that the quality specifications are met. These are done in Quality Control certified laboratories following the European pharmacopoeia, the official document that regulates the European pharmaceutical industry.

To conduct these analyses, it is necessary to sample the materials upon arrival at the unit. A step that is necessary for each storage unit (SU) per batch that, when in large quantities, can hold the laboratory team until complete. This can cost the entire process, from the arrival of the raw materials to the product production, a lot of time and with many samples to test, the risk of errors in the analyses rises.

Even before the sampling, the Raman Spectroscopy technique allows for fewer samples and reduces the number of tests necessary. This results in a lower time spent on sampling and analyses and even prevents contaminations by removing the necessity of opening all the SU as it does not require direct contact with the product.

The application of this technique shows a reduction of around 65% of the time taken by the sampling process and in the amount of laboratory analysis of a product where the technique was applied. This shows a significant improvement in productivity by reducing the overall time necessary to process material before ready to use in production.

KEYWORDS**:** Raman Spectroscopy, Raw Materials, Good Manufacturing Practices, Quality Control, Pharmaceutical Industry.

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## LIST OF ACRONYMS AND ABBREVIATIONS

API – Active Pharmaceutical Ingredient

CFR - Code of Federal Regulations

CoA – Certificate of Analyses

EMA - European Medicines Agency

EP – European Pharmacopeia

GMPs – Good Manufacturing Practices

GLPs – Good Laboratory Practices

ICH – International Council for Harmonisation

IR – Infrared

MAA – Marketing Authorisation Applications

QA – Quality Assurance

QC – Quality Control

OOS – Out-of-Specification

# 1. INTRODUCTION

The present internship took place in *Generis Farmacêutica S.A.*, more specifically in the company's Quality Control department, for the curricular internship of the master's degree in Biological and Chemical Engineering of the Barreiro School of Technology, Polytechnic Institute of Setúbal (ESTBarreiro/IPS). Beginning on the 14th of March and ending on the 19th of July 2022.

Quality control includes specific analyses of samples retrieved from the arrived materials to guarantee that the raw materials used in the production of pharmaceuticals comply with the quality standards imposed by the respective authorities. One of the first analyses carried out is the identification of products by Raman spectroscopy even before the acceptance for laboratory analysis of the product guaranteeing that this is indeed the described product.

This technique is also to be implemented in bulk products, produced externally but packed in the facility, giving a form of identification to the active pharmaceutical ingredient, being them in the form of a tab or inside a capsule.

The objective of implementing this technique is the possibility of reducing the time spent on sampling, reducing the risk of contamination by minimizing the direct contact with the materials, the preliminary identification of the components at the arrival and external bulk and the reduction of the amount of time spent on quality analyses.

Implementing this process affects various activities in the quality control department such as the sampling process, intake, analysis, and verification, positively impacting the productivity and money saved by the company.

## 1.1 The Company

*Generis Farmacêutica S.A.* (Fig. 1) is a Portuguese pharmaceutical laboratory that specializes in the generic pharmaceuticals market.

Figure 1 – Company Logo [1].

Founded in 2002 and acquiring its first installations in 2006 in Venda Nova, the company quickly grew to become the biggest portfolio in Portugal and one of the biggest in Europe [1].

Generis is now a part of the Aurobindo group (Fig. 2) having a total of twenty-five factories and 7 I&D installations around the world, being the number one generic drug company at a national level. The group is scattered across 34 countries on 4 different continents [1], [2].

Figure 2 – Aurobindo Group Logo [1].

## 1.2 COMMERCIAL ACTIVITY

Its portfolio reaches over 85% of the health and care areas, made up of 1492 prescription drugs, 254 hospital-use products and 67 health products. They produce antibiotics, dermatological, oncological, anti-retroviral, antiallergics, and more [2].

## 1.3 INDUSTRIAL ACTIVITY

The industrial unit opened in Venda Nova, Amadora, Portugal in 2006 (Fig. 3), and has the capacity to produce thirty million drug packages annually. It has an area of 8.125 square meters, divided between production, analyses laboratory, administration, and warehouse, and has direct access to the main transport routes for easy intake of raw materials and outtake of the finished product. It also has a development area for batch production tests with equipment at a scale of 1/10 allowing for a low-risk scale-up [3].


Figure 3 – Generis Industrial Unit in Amadora [1].

## 1.4 EXPORTS

Generis is present in the Angola, Cabo Verde, Mozambique, and Macau markets, and in the middle Este in Lebanon, Libya, and Iraq through strategic partnerships with agents and local distributors. On the American and Asian continents, there are also already registered products in addition to exporting to 21 European countries (Fig. 4) [4].

Figure 4 – Generis exports targeted countries [4].

## 1.5 QUALITY CONTROL UNIT

Generis Quality Control Unit is composed of a fully equipped quality control laboratory prepared for stability testing following the International Council for Harmonisation (ICH) regulations, a separate microbiology laboratory for examination and identification of microorganisms to monitor and avoid the existence of contaminated products, and a sampling bay for initial quality evaluation and sampling at products arrival.

The Unit is also separated into different teams responsible for all the aspects needed to work. These serve as a quality control point for each team's aspect.

- Sampling team,
- Sample Flow team,
- Raw materials team,
- Finished products team,
- New products team,
- Stabilities team,
- Microbiology team.

### 1.5.1 SAMPLING BAY

The Sampling Bay is where a first inspection of the newly arrived product is performed and if this passes it is then sampled and sent to be thoroughly analysed. This reduces time spent on

analysis if the product has clear quality defects being rejected and sent back to the supplier with the proper details of rejection.

It also has chambers ready for sample gathering in aseptic conditions preventing any outside contaminations for both the material and the samples retrieved. These samples are then sent for analysis in the Quality Control QC and Microbiology Laboratories, and this will determine if the arrived materials are accepted and sent for production or not.

### 1.5.2 Quality Control Laboratory

The QC Laboratory's primary functions are sample and batch analysis. This is fully equipped and ready for product quality analysis meeting the demands of International Pharmacopoeias [5], [6].

This is the main laboratory where the analysis teams can verify the quality of the products received and produced including stabilities and the test of new products.

### 1.5.3 Microbiology Laboratory

In the Microbiology Laboratory, the samples are tested for biological contaminations. This laboratory is separated to ensure full control over what comes inside preventing any external factors that would affect the results from these products. The laboratory ensures fully aseptic conditions to rigorously test the presence of microorganisms in the tested products.

# 2. OBJECTIVES

The objectives for this curricular internship were to learn and train the pharmaceutical industry notations such as the Good Manufactory Practices (GMPs) and Good Laboratory Practices (GLPs), to create and implement the co-validation Raman method to reduce time spent in sampling and quantity of required analyses and to participate in physicochemical laboratory analysis in the quality control department while comprehending the practical application of the different techniques and procedures.

# 3. INTERNSHIP CHRONOGRAM

The internship lasted a total of 600 hours from the 14th of March 2022 to the 19th of July 2022.

The tasks planned for this period were the following:

- Task A – Quality Control Unit Initial Training
- Task B – Raw Materials Raman Spectra Gathering
- Task C – Product Certificates and Infrared Spectra Gathering
- Task D – Quality Assurance Documentation Creation
- Task E – Thesis Writing

Table 1 - Internship chronogram from the 14th of March 2022 to the 19th of July 2022.

| | Weeks | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Tasks** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| **A** | | | | | | | | | | | | | | | | | | | |
| **B** | | | | | | | | | | | | | | | | | | | |
| **C** | | | | | | | | | | | | | | | | | | | |
| **D** | | | | | | | | | | | | | | | | | | | |
| **E** | | | | | | | | | | | | | | | | | | | |

# 4. THEORETICAL FUNDAMENTS

## 4.1 QUALITY ASSURANCE

Quality assurance is a wide notion that covers all matters impacting the trust that the standards for quality in a product are met for both the management internally and the consumer, authorities, and others externally. [7]

The Pharmaceutical Industry upholds high standards of quality assurance throughout the development, production, and control of pharmaceuticals, ensuring their stability, effectiveness, and safety. [7]

For this, it is necessary to assure control of the product's quality throughout every step of its production, from the arrival of raw material to the final product's release including storage, manufacture, and packaging. [7]

## 4.2 QUALITY CONTROL

Quality Control is the department responsible for sampling, analysing, and establishing specifications for analytical methods and applications while always assuring appropriate documentation using up-to-date practices [7], [8].

For this, large firms need a separate department specifically for quality control that ensures:

- The adoption and compliance of Good Manufacturing Practices (GMPs) and Good Laboratory Practices (GLPs).
- The management responsibilities are written and clearly defined.
- The verification of the quality of pharmaceuticals by a qualified person under the Marketing Authorisation Applications (MAA) requirements.
- The adequate storage, distribution, and handling of pharmaceuticals throughout their designated validity period.
- Intervention when an "Out-of-Specification" (OOS) occurs, within the scope of the opening of an investigation, conducting and coordinating the same.
- The performing and updating of technical procedures and product standards.

### 4.2.1 QUALITY CONTROL DEPARTMENT

Quality Control can be divided into two sectors, the microbiology and the physiochemistry sectors, and the latter in turn, is divided into teams responsible for the analysis of all products that require quality control like Raw Materials (RM), Packaging Materials (PM), and Bulk Products (BP).

The associated Control Instruction, Sampling Instruction, and Sample Labels for each RM, PM and BP are stored in SAP, the management software used by *Generis Farmacêutica S.A.* The software automatically generates an internal batch number for each product at arrival and reanalysis products.

The specifications for the preparation of each test follow the respective monographs in Pharmacopoeia. This has all the information about the product, tests required to perform, and results deemed acceptable for quality apart from a few regulated in-house modifications that never deviate far from these standards [5], [6].

For each analysis, whenever a device is used the analyst must record its use in the Logbook, noting the day, the product analysed and the respective batch number, to create a usage history. This ensures control for all devices and an easy backtrack if ever necessary.

All results from the analyses are entered in SAP verifying that it complies with the associated specifications. From here the return can be a positive Certificate of Analysis (CoA), printed for a product that meets all the quality standards required to test, or a negative CoA for a product that doesn't meet the quality standards for one or more tests with the highlighted respective value Out of Specification (OOS).

### 4.2.2 Good Manufacturing Practices

Good manufacturing practices are a part of quality assurance. This ensures that products are produced and controlled consistently to the quality standard appropriate to their intended use and as required by the Marketing Authorization or product specification. GMP applies both to production and quality control [9], [10].

In the European Union (EU), the European Medicines Agency (EMA) coordinates inspections to verify compliance with these standards and plays a key role in harmonising GMP activities. The rules Governing Medicinal Products in the EU:

- EudraLex Volume 4 – EU Guidelines to GMP medicinal products for human and veterinary use [10].

Standard GMP requirements There are requirements for all areas involved in pharmaceutical products manufacture:

- Incoming materials management,
- Process equipment and facilities,
- Production,
- Analytical Laboratory controls,
- Batch release,
- Change control requirements,
- Documentation requirements Record-keeping.

### 4.2.3 Good Laboratory Practices

"The principles of Good Laboratory Practice (GLP) define a set of rules and criteria for a quality system concerned with the organisational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, reported, and archived." [11].

These are regulations published in the Code of Federal Regulations (21 CFR Part 58 [12]) and have nothing to do with manufacturing products instead both co-exist.

### 4.3 MEDICINE AND GENERIC MEDICINE

A medicine product is a technically prepared product, containing one or more drugs associated with other substances, for prophylactic, curative, palliative or diagnostic purposes. It has proven diligence, safety, and quality by undergoing medical trials before its release to the consumer market [13]–[15].

The pharmaceutical forms can be presented in deferent physical states such as liquids (solution, syrups, elixirs, suspensions, emulsions, drops, injectables, etc.), solids (tabs, capsules, powders, gums, granulates, suppositories, etc.), semi-solids (ointments, creams, pastes, etc.) e gasses (aerosols e inhalants) [16], [17].

According to the European Medicines Agency (EMA), a generic medicine is developed with the same qualitative composition in active substances, the same pharmaceutical form and dosage as a brand-name medicine that has already been authorised. A bioequivalence test is performed to demonstrate that in the human organism the same quantity of active substances exists for each generic medicine and reference medicine demonstrating that the actuates in the same form. For this, it has the same therapeutic application as its reference brand medicine [13].

Its authorisation is based on efficacy and safety data from studies on the authorised medicine. A company can only market generic drugs when the original drug's 10-year exclusivity period has ended [18].

All the medicines, including generic medicines, are manufactured in facilities that follow the regulations and that are periodically inspected by the proper authorities [13], [14].

### 4.4 RAW MATERIALS

In the pharmaceutical area, the raw materials utilized in the production of medicine can be of two types: Active Pharmaceutical Ingredients (APIs) and Excipients [19]–[21].

**APIs** are the substance that gives the intended beneficial health effect to the consumer. There are many different APIs used for the creation of pharmaceutical drugs, differing in the benefit given, dosage utilized and others [19], [21].

A placebo replicates the appearance of a pharmaceutical drug but instead of an API, it is used as an Inactive Ingredient such as starch or sugar.

**Excipients** are the components used to achieve the final pharmaceutical form intended to facilitate its production, taking and conservation [19]–[21].

There are 10 types of Excipients based on the intended use: **Anti-adherents** used to reduce the adhesion between the granules, **Binders** hold the ingredients in a tablet form, **Coatings** prevent ingredient deterioration by moisture, **Disintegrants** dissolve when wet causing the tablet to break apart, **Fillers/Diluents** fill out the size of the tablet, **Flavours** used to mask unpleasant taste, **Colours** improve the appearance of the product, **Glidants** promote powder flow by reducing particle friction, **Lubricants** prevent ingredients from clumping together and sticking to the tablet and **Preservatives** that preserve the formulation [20].

## 4.5 BULK PRODUCTS

The medicines produced in various forms are called bulk and these also undergo different analyses to guarantee that their quality, stability, and efficacy standards are met.

Some products arrive already as final from a client (a different bulk manufacturer) and are only packed in the facility. These are called external bulk.

## 4.6 SPECTROSCOPY

Spectroscopy is the study of the interaction of light and other radiation with matter. This concept can be used in the visualisation or even identification of elements and compounds by capturing/measuring the radiant energy absorbed or emitted at a determined wavelength of the electromagnetic spectrum on excitation by an external energy source [22], [23].

This technique has been decisive in the development of the most fundamental theories in physics. It has been used in many areas of science and technology. Some well-known examples include magnetic resonance imaging (MRI), to visualize the internal soft tissue of the body with unprecedented resolution, microwave spectroscopy, used to discover the three-degree blackbody radiation, the remnant of the big bang from which the universe is thought to have originated, the internal structure of the proton and neutron and the state of the early universe up to the first thousandth of a second of its existence, the constituents of distant stars, intergalactic molecules, and even the primordial abundance of the elements before the formation of the first stars can also be determined by optical, radio, and X-ray spectroscopy. Today, spectroscopy is most used to determine the chemical composition and physical structure of various compounds [24], [25].

The theory is based on the concept that light is made up of different wavelengths, each corresponding to a different frequency, and every element in the periodic table emits or absorbs different frequencies forming a consistent and unique light spectrum. The big advantage is the fact that any part of the electromagnetic spectrum, from infrared to ultraviolet, can be used to analyse a sample making it possible to gather different properties and overcome issues with the readings [22], [26].

Another big advantage is the fact that it is mostly a non-destructive technique and, depending on the application, even non-intrusive by removing the need for direct contact with the subject as long as the wave's energy is low enough not to interact significantly with it [22].

Spectroscopic techniques are very sensitive. Individual atoms and even different isotopes of the same atom can be detected among $10^{20}$ or more atoms of different species. Small amounts of impurities or contaminants are often detected most effectively by spectroscopic methods. Certain types of microwaves, optical, and gamma-ray spectroscopies can measure infinitesimal frequency shifts in narrow spectroscopic lines. Ultra-high-resolution laser techniques can detect frequency changes as small as one in $10^{15}$ of the frequency measured. Because of this sensitivity, the most accurate physical measurements were frequency measurements [22], [26].

### 4.6.1. RAMAN SPECTROSCOPY

Raman Spectroscopy is the study of the frequency shift that occurs in the light scattered on a sample. An unknown sample of material is illuminated with monochromatic (single wavelength or single frequency) laser light, which can be absorbed, transmitted, reflected, or scattered by the sample. The scattered can return with the same frequency due to elastic collisions, called Rayleigh scattered light, and with different frequencies due to inelastic collisions, called Raman scattered light. These correspond to the vibrational frequencies of the molecular bonds of the sample, and it is similar to what is observed in infrared spectroscopy, which yields similar yet complementary information (Fig. 5) [27]–[29].

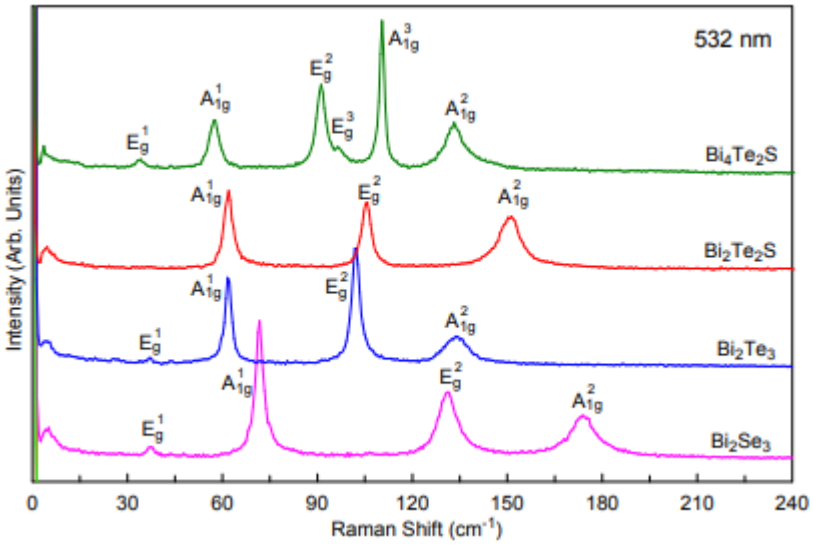


Figure 5 – Example of Raman spectra of $Bi_2Se_3$, $Bi_2Te_3$, $Bi_2Te_2S$ and $Bi_4Te_2S$ [43].

The photons from the scattered light, when returned to a receptor, can have a lower frequency, called Stokes Raman scattering, or a higher frequency, called anti-stokes Raman frequency. [30] This shift in the frequency depends on the chemical composition of the sample. The intensity of Raman scattering is proportional to the magnitude of the change in the molecular polarization (Fig. 6) [27], [29].

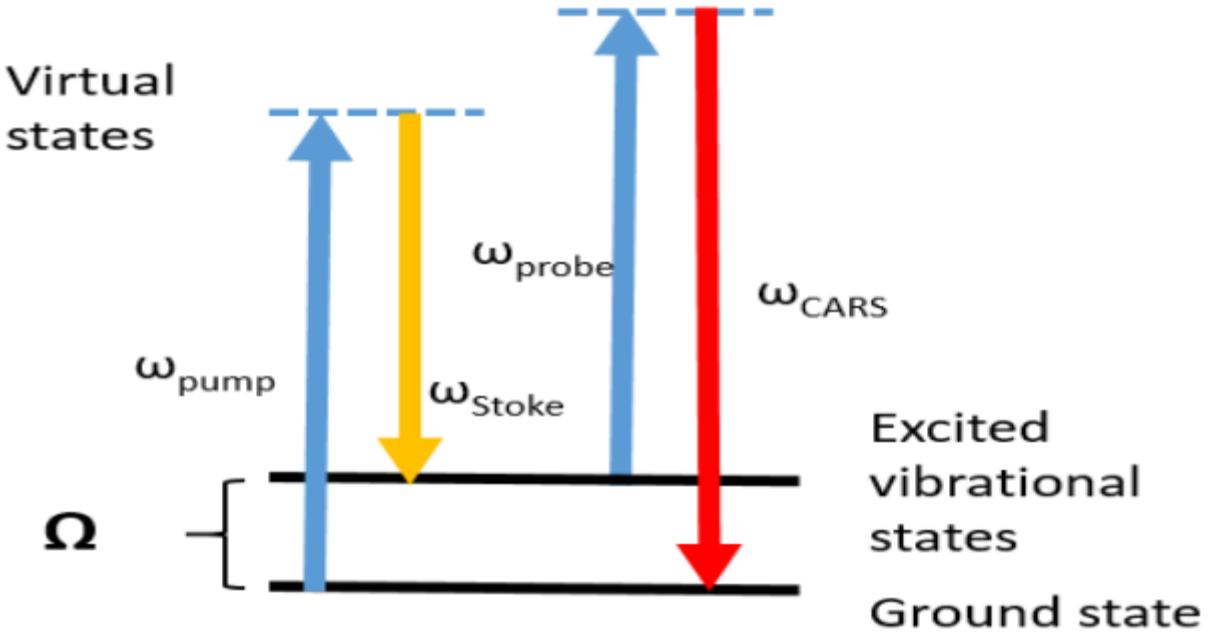


Figure 6 - Diagram of the coherent anti-Stokes Raman scattering (CARS) process [32].

This phenomenon was first observed by Chandrasekhara Venkata Raman in 1928 who received the Noble prize in physics for this work in 1930 [31].

Spontaneous Raman scattering is typically very weak, as a result, for many years the main difficulty in collecting Raman spectra was separating the weak inelastically scattered light from the intense Rayleigh scattered laser light (laser rejection) [28], [32].

Historically, Raman spectrometers used holographic gratings and multiple scattering stages to achieve a high degree of laser rejection. Modern instrumentation almost universally employs notch or edge filters for laser rejection. Dispersive single-stage spectrographs (axial transmissive (AT) or Czerny–Turner (CT) monochromators) paired with CCD detectors are most common although Fourier transform (FT) spectrometers are also common for use with near IR lasers. Nowadays, Raman systems use a combination of autofocus devices, and automated data acquisition and analysis procedures to acquire spectra from hundreds of samples sequentially [27], [29].

There are many other variations of Raman spectroscopy including surface-enhanced Raman, resonance Raman, tip-enhanced Raman, polarized Raman, stimulated Raman, transmission Raman, spatially offset Raman, and hyper Raman [29], [32]–[35].

One disadvantage of this technique is the interference by fluorescent samples. This has been resolved by incorporating excitation lasers of different wavelengths which can be changed to near infrared to reduce the fluorescence of problematic samples.

One of the advantages of Raman is the possibility to separately identify polymorphs. These are solid materials in different structural forms making them hard to identify. Their formation can lead to differences in the chemical and biological properties of drug candidates that, in turn, impact their quality control, licensing and patent protection requirements [36]. Raman spectroscopy is proven capable of separately detecting these polymorphisms making it an especially useful technique in the pharmaceutical industry. Some of the polymorphisms proven to be separately detected by Raman spectroscopy are acetaminophen and fenofibrate (Fig. 7) [37].

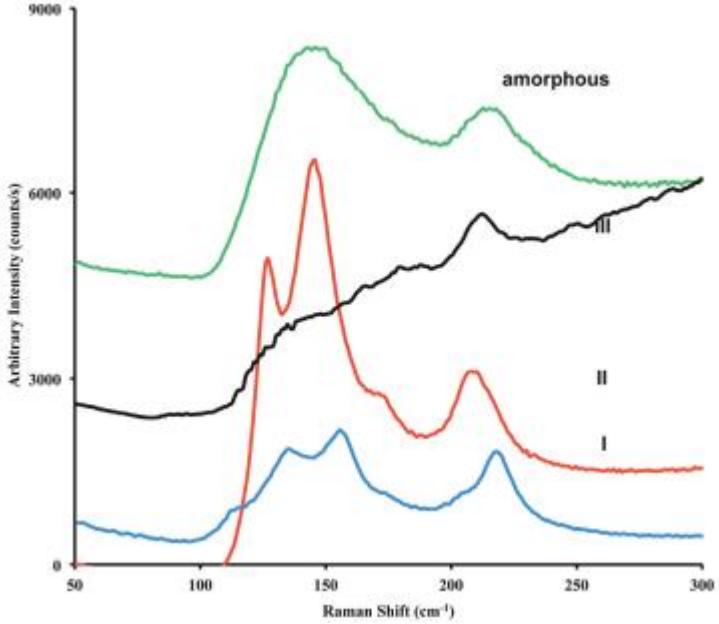


Figure 7 - The low frequency Raman spectra of the amorphous and crystalline phases I, II and III polymorphs of acetaminophen [37]

The figure above (Fig. 7) is an example demonstrating that different polymorphs have different Raman signatures making it possible to use this technique in the identification of these [37].

### 4.6.2. Raman Spectroscopy vs. Infrared Spectroscopy

Although there is a similarity in the theory behind Raman and Infrared (IR) spectroscopy these techniques differ in several aspects. Both are types of vibrational spectroscopy, which deal with the same vibrational energy levels of the material, however, each one is based on different ways of interacting with the studied object results are driven by different principles. In general, Raman scattering depends on the change in polarization, while infrared spectroscopy mainly depends on the change in dipole moments [32].

These techniques can also have different pros and cons making them complementary, one example being that Raman spectroscopy can identify different components in sample mixtures

much easier than IR spectroscopy due to its narrower bandwidth. IR signal is produced upon IR light absorption and since this technique uses long wavelength excitation, the corresponding spatial resolution is low. This resolution was reported to be in the range of 2.5-25 μm [32].

Another problem regarding IR spectroscopy is due to water absorption. Strong water absorption impedes the use of IR use in bio-imaging, archaeology and other studies that contain water or moisture in its studied structure. Such problems were avoided in Raman spectroscopy [32], [37].

Raman spectroscopy can use an excitation laser source that works in the visible or near IR spectral range. Also, Raman cross section scattering is lower than IR absorption making it not affected by the presence of air, water or glass making it possible to adapt to a non-destructive and contamination-free technique [32], [37].

### 4.6.3. TRUSCAN RM

The Thermo Scientific TruScan RM analyser is a handheld Raman spectroscopy used in material identification commonly in the pharmaceutical areas. It can acquire the product's spectrum and perform the statistical assay automatically, reducing the need for user input and making it easy to use by non-technical staff (Fig. 8) [38].


Figure 8 – Thermo Scientific Truscan RM device [44].

The device has a spectrum range of 250 to 2875 Raman Shift ($cm^{-1}$) and a spectral resolution of 8 to 10.5 $cm^{-1}$. It is composed of a class IIIB laser diode that emits a 250-mW beam at 785 nm and an 18 mm focal length lens used to concentrate the energy on a sample. The beam diameter at the plane of the focal lens is approximately 9.6 mm [38].

Depending on the product characteristics, this process can take around 1 minute per SU analysed reducing the time spent on sampling by $t * n - (t * (\sqrt{n} + 1) + n)$ (time spent moving pallets excluded, time spent applying the technique), being t the duration of the sampling process and *n* the number of SU's from the batch rounded to the nearest whole number.

**E.g.:** For a batch with 60 SU and a sampling time of 5 minutes per SU, the time saved by utilizing the technique would be 195 minutes (300 minutes taken without and 105 with the technique), a 65% reduction.

It comes with two different nose cones (ends), one normal (PnN) and the other blunt (PnC) that give similar readings at different speeds both having different tendencies to fluorescence errors depending on the characteristics of the material analysed such as colour and reflectance [38].

The devices gather the product's spectra (signatures) that are later organized in different groups for each product (called methods) respective to the product. These methods should have a minimum of three signatures per product for repeatability. With a finished products database, it can perform an identification analysis based on statistics to compare with the already read material returning a "Pass" or "Fail" giving us the material's chemical identity (Fig. 9) [38], [39].


Figure 9 - Example of both the "Pass" and "Fail" screen and respective match using the TruScan RM [39].

#### 4.6.3.1. PROBABILISTIC APPROACH

Using statistical testing, it is possible to predict the outcome of action with a narrow margin of error. This is performed to test the hypotheses of a certain event, returning the chance of its occurrence [40], [41].

The *p*-value in significance testing is the chance obtained from it and when very low, frequently less than 0.05, it is assumed that the event should not occur. The opposite is also true, if the p-value is greater than this value, it's likely to observe this outcome. In other words, the observation is highly unlikely to be the result of random chance [40], [41].

TruScan not only acquires the Raman spectrum of the material of interest but also determines the uncertainty of that measurement in real time. This information gives us how repeatable and reliable we expect that measured spectrum to be over similar or even different sampling conditions. In statistical terms, uncertainty refers to standard deviation (Fig. 10) [39].

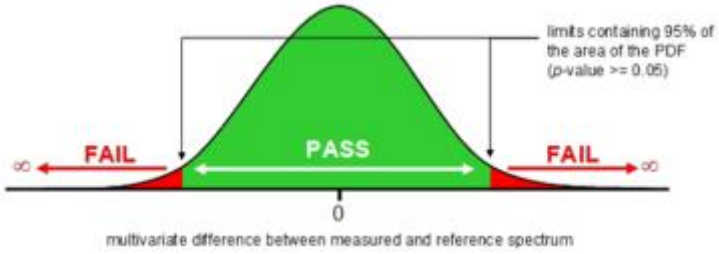


Figure 10 - TruScan RM pass/fail univariate t-distribution [39].

To calculate the uncertainty, a P-Value Approach Statistical test is automatically performed on the measurement made to the sample, given factors such as the measurement settings (e.g., exposure time and the number of scans or sweeps), environmental properties (e.g., temperature, dark current) and the properties of the sample of itself (e.g., Raman cross section, absorbance, refractive index, etc.) [39].

The spectrum specifications result in approximately 2000-dimensional spectral data, with the p-value test conducted in all dimensions simultaneously. TruScan tests if the measurement of the test material is statistically consistent with the measurement of the reference material. If there is no significant difference between test and reference measurements, falling within the limits corresponding to a p-value of 0.05, the sample measurement is considered consistent with the reference spectrum, within the uncertainty of the measurement, and the device will report a positive match. If the difference falls outside these limits (p-value ≤ 0.05), the device will report no match [38], [39].

The probability curve is drawn by the software during method development and its shape is a function of easily modelled sources of uncertainty. Since the software models the uncertainty directly, there is no calibration or user modelling involved with method development. A single reference spectrum typically suffices for method development with bulk materials because the physical properties of the sample (e.g., particle size, packing density, humidity/water) have minimal influence on the Raman spectra TruScan acquires, and the remaining sources of variability are modelled directly by the embedded analysis [39].

If more than one reference spectrum is used for a TruScan RM method, the test is repeated for each reference spectrum, and if the outcome of any comparison passes the test, then the method will pass as well. That is, the measurement of the material was consistent with at least one of the measurements of the reference materials. The analysis does not average the reference spectra together and performs a test of equivalence using the average spectrum (Fig. 11).

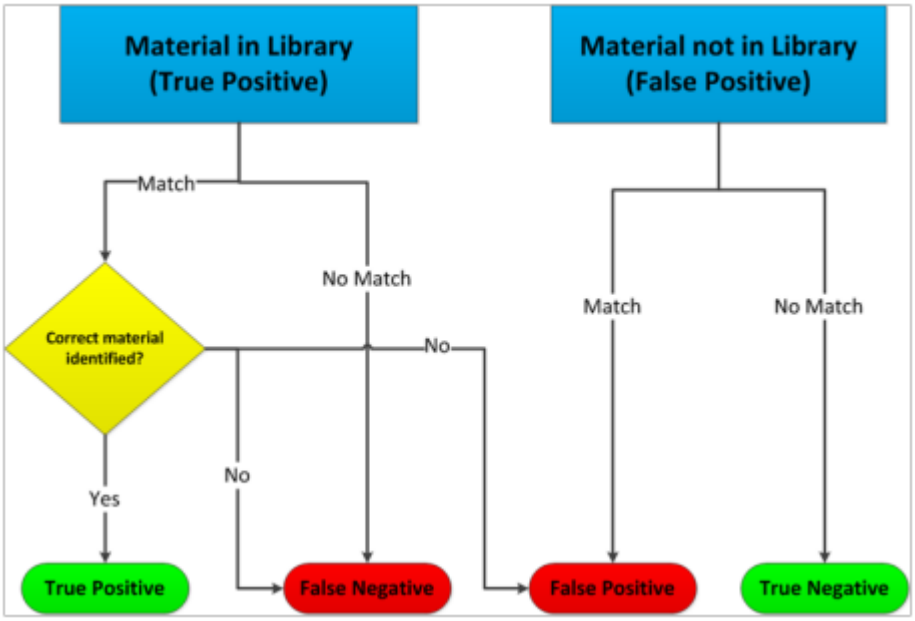


Figure 11 - Outcome tree for decision making based on a qualitative result [39].

#### 4.6.3.2. SELECTIVITY

The device has a built-in function to test a product's selectivity with the entirety of its library. The test is performed to ensure that the measurements of a product's spectrums are statistically different from other products ensuring that no product has similar values that could lead to a faulty reading [38].

During the validation procedure, the selectivity of the database must be ensured, which allows the materials to be identified as sufficiently separated from others in the database [42].

#### 4.6.3.3. TECHNIQUE VALIDATION

For the implementation of a technique, it is required the validation of the same to confirm that this is a valid technique and that its application is correctly described to avoid any errors in its application. This is done by a Quality Assurance department that oversees every technique and instrument utilized in the company preventing discrepancies in readings performed by the inconsistency of methodology and guaranteeing that the quality required in the analysis and production is achieved [42].

# 5. Procedure

For the application of Raman spectroscopy, it was necessary the creation of an in-house database for all the materials that can benefit from this technique. It is also necessary to validate any reading performed for this database to ensure the procedure is rigorously controlled so any error performed or abnormal value gathered that can impact the subsequent analyses can be detected and studied and corrected.

## 5.1 Data Gathering

For the creation of a database storing the readings (also called product signatures) and information for each product, a previously prepared excel table was modified to accommodate all the data and easily sort the previously gathered batch information. This table includes the materials for the technique, their SAP code used for tracking in-house, the batch number and respective external batch and test order ID, the type of sample used (In Sampling Cup, Sampling Bag, Original Package or Vial), Type of ends used (PnN or PnC), Test ID (Signature) and test taker and Documentation IDs. This table already had a template and some information present previously gathered that was then updated.

For each product, signatures from a minimum of 3 batches were required following the scientific method and the recommendations from the manufacturer. Each batch also required a specific IV spectrum (previously performed) and Certificate of Analyses (CoA) from both the company and the supplier.

## 5.2 IR Spectrum and Certificate of Analysis

The implementation of this technique requires a database of previously tested products ensuring that these are within specification. Both the IR spectra and Certificate of Analysis (CoA's) were already available from previous tests and are required to annex has information for each batch of each product utilised.

The API batches used in the creation of the database were already analysed in the laboratory and had their respective IR spectrum stored in-house for a determined amount of time, depending on the product, as mandatory by European regulations. These included the comparison between the sample spectrum and the standard spectrum.

The two CoA's needed were from the manufacturer, which arrives together with the respective batch, and a company's CoA that is released after the successful result of each test required

on the product. Both have information on the ID, characteristics and test results for the product and must be signed by competent personnel.

## 5.3 TRUSCAN RM

The TruScan RM analyser (SN TM4365, June 2014) is a handheld Raman analyser for material identification from Thermo Scientific. It is the device used by the company to perform rapid material identification to decrease sampling costs and time spent on inventory while preventing the contamination of the product by performing a point-and-shoot analysis of the compounds through the sealed packaging removing the necessity to expose the contents.

### 5.3.1 METHODS GATHERING

For comparison of the spectrums gathered from a run, it is necessary to have a database for comparison. Thermo Scientific has a built-in standard database of previously measured spectrums of a variety of raw materials and products, but it was chosen to create an inside database using samples stored in-house.

To follow the Scientific method, these methods were created using 3 different batches from each product. This ensures that there is little possibility of an error occurring in these readings.

The Spectra gathering can be done using either Sampling Cups, Sampling Bags, the Original Package, or a Vial to perform the measurement.

### 5.3.2 SELECTIVITY TESTE

When all the spectra for a product are gathered in the software in their corresponding method, a selectivity test is performed using the device. This test can be performed on any batch from the method's product and the expected result should be a match for the exact method and no other product.

A similarity with an equal product from a different supplier or a weak similarity with a different product with a justification for this similarity can be accepted.

## 5.4 EXTERNAL BULK EXPERIMENT

The utilization of Raman spectroscopy for finished products with mixtures of components was not completely tested so an experiment on these products was performed to analyse if the application of the technique in these is feasible by the company.

Besides the existence of mixtures, the difference in forms (tabs, capsules, etc.) shapes, colours, presence or not of coatings, and content of capsules (powder, liquid, etc.) make for some of the variables tested in this experiment ensure the results from the readings of the same product are the same regardless of the different variables. This experiment shows if any

of these variables interfere with the device's laser making it not possible to apply the technique if it does.

In the studied products, readings were performed in three different batches (When present, the remaining wait for the arrival of new batches (Component B and Component C)) of each of 5 different capsules assuring the result's repeatability for each. These readings were performed with both ends, the normal end (PnN) and conic end (PnC), and with the capsule's content separated into a vial.

- Component A: liquid content, yellow capsule.
- Component B: solid content, transparent body, green head.
- Component C: solid content, yellow body, red head.
- Component D: liquid content, blue capsule.
- Component E: liquid content, white capsule.

In capsules with more than a colour (Component B and Component C), it also performed readings with both ends on each colour.

There were two types of products to consider, liquid-content capsules and solid-content capsules, so different extraction were performed for both types with a previous reading of the complete capsule.

### 5.4.1 Liquid Content Capsules Extraction

For these (Component A, Component D and Component E), the extraction was performed using a syringe and then deposited in an HPLC vial. The content from one capsule was enough to acquire a reading of the Raman spectra of each batch.

### 5.4.2 Solid Content Capsules Extraction

For these (Component B and Component C), the extraction was simpler being only necessary to separate the capsule's head and body and the deposition of its content to an HPLC vial. The content from one capsule per batch was also enough to acquire its Raman spectra for these.

### 5.4.3 HPLC Vials Readings

The HPLC vials have a diameter considerably lower than the device's adapter having the readings needed to be performed in a way that no gap is present between the device's wall from the laser origin side and the vial, reducing so any additional interference.

For this, the reading was performed with the device angled enough to make the vial "fall" on the adapter wall without needing any manual support to avoid sample movements during readings. This method is performed equally for every product.

### 5.4.4 Empty Capsule Reading

The empty capsule reading was tested to understand if it would interfere with the content spectrum during reading, but it was only possible to obtain one reading from Component D's capsule due to the Raman signal being too low to obtain results from the remaining.

With the result obtained it was possible to make conclusions without the need for the remaining readings.

### 5.4.5 Quality Assurance Documentation

All the procedures, results and conclusions taken were compiled to report that serves as a company's official document assuring the methodology feasibility. As every technique performed by the company requires a document associated, this was required as a step of verification that ensures the validity of the study and its results.

This document will be reviewed by the Quality Assurance department and if approved it is later publicised in the company's internal document system making the technique ready to use in the reported products.

# 6. RESULTS AND DISCUSSION

## 6.1 EXTERNAL BULK EXPERIMENT

### 6.1.1 HPLC Vial

The HPLC vials have an associated interference that makes their use for the spectra acquisition for method creation less viable. Even so, it is possible to use them only for comparison in the study of capsule interference. An empty vial was tested (Fig. 12).

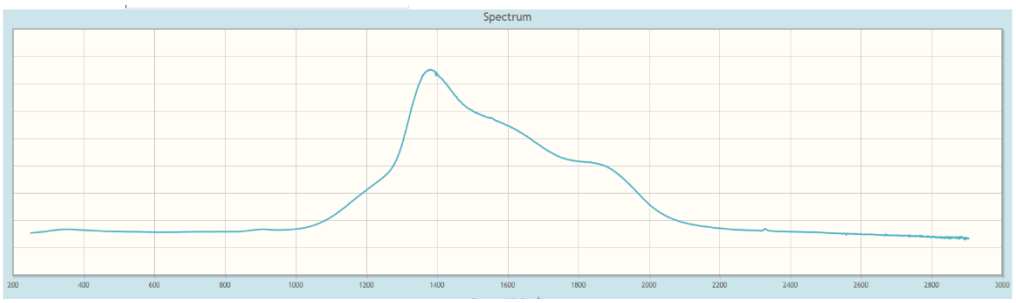


Figure 12 – HPLC vial Spectrum.

With this spectrum, it is possible to understand the HPLC vial Raman profile making it possible to discard this interference when observing the results. The peak observed in the figure (Fig. 12) is considered an interference in the reading and the vial should be used to perform this test subsequently, but because the objective of this experiment was to understand if the capsule causes interference in the readings, it can be used to compare the signatures from the content both in and out its capsules noting that this profile while being present in the content in vial readings and needs to be discarded.

With all the readings gathered, a comparison was made between full capsules (without any manipulation) and their contents in the vial to understand if the capsule interfered with the readings of its content.

For all the products it was possible to find the same spikes between readings of full capsules and in vials, some highlighted in the figures below (Fig. 13 to 17), only differentiated by their intensity and vial interference (this discarded (Fig. 12)).

**NOTE:** *As the results for every batch of every product are similar, the remaining are in the annexe.*

### 6.2 **BULK**

### 6.2.1 Component A

In the figure (Fig. 13) we can observe that the content is identified both in the capsule and in the HPLC vial, by its equal positioning of the discovered spikes.

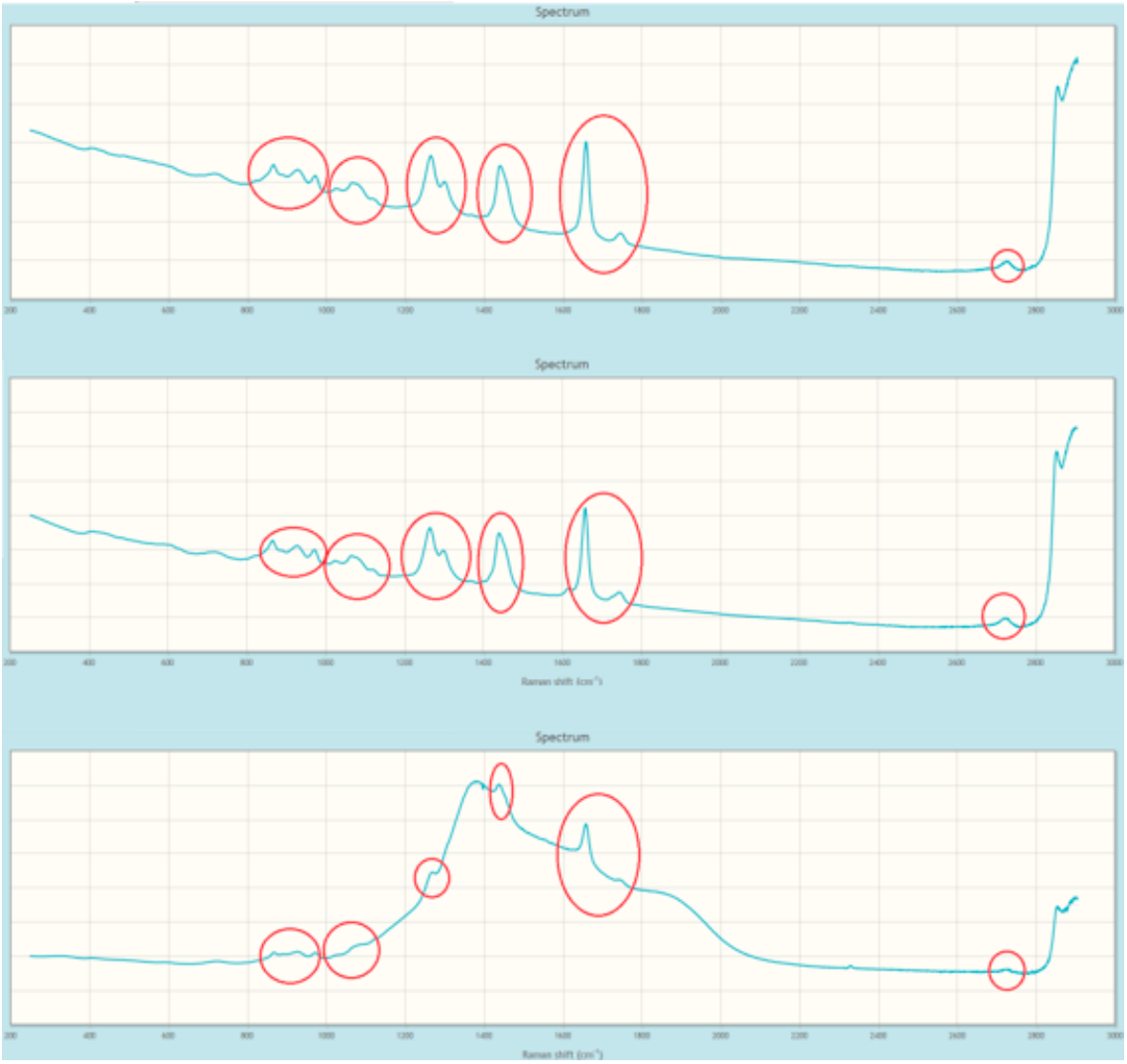


Figure 13 - Raman Spectra of the Component A. On top the reading with PnN on a full capsule, in the middle reading with PnC on a full capsule and bellow reading of its content in vial.

It is possible to conclude that the capsule does not impede the reading of its content and, in case interference is present, it is not noticeable nor comparable to the vial interference.

### 6.2.2 Component B

This product has a capsule with more than one colour, a transparent body and a green head. Because of this, the spectra from both colours were obtained and compared using both ends (Fig. 14).

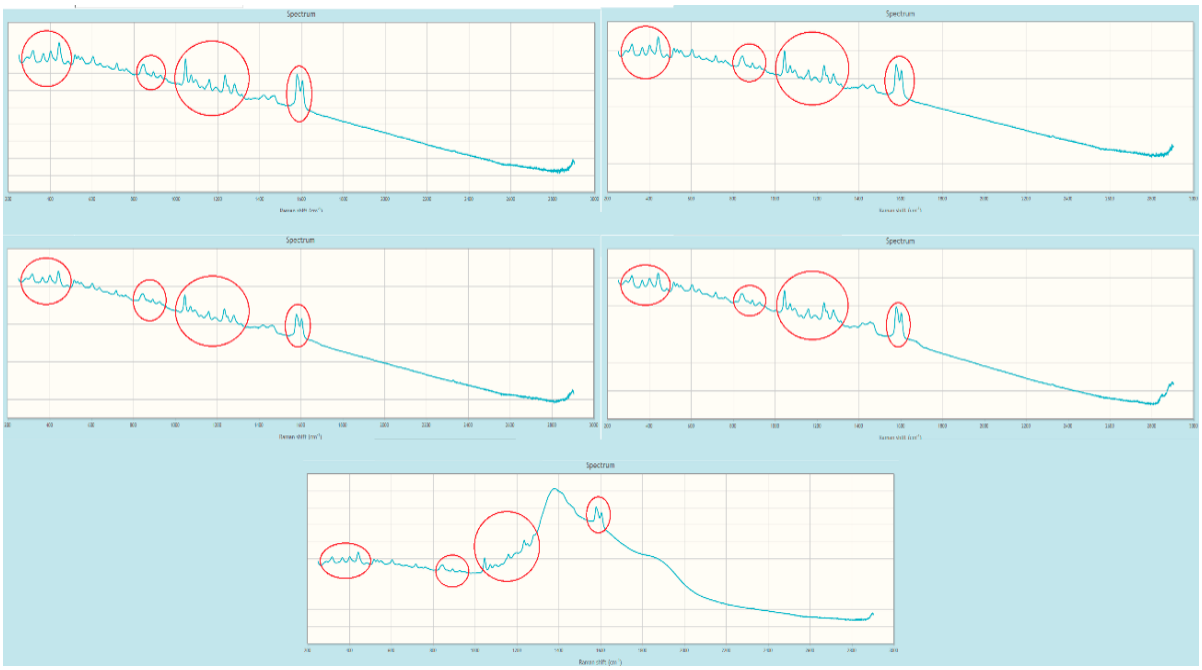


Figure 14 – Raman spectra of the Component B. On top reading of transparent body (PnN to the left and PnC to the right) on full capsule, in the middle reading of the green head (PnN to the left and PnC to the right) on full capsule and bellow reading of content in vial

In this figure (Fig. 14) the vial interference is more noticeable in the zone it is more expressed (approx. 1300 a 1500 $cm^{-1}$) not being possible to visualize the peaks with ease, but product identification is still possible.

Also, noticeable a slight difference in pike intensity read in the different colours. Although this exists, this interference doesn't influence the identification of the products and can be disregarded.

### 6.2.3 COMPONENT C

Once more it is possible to observe the same results gathered in the previous products and having the capsule in two different colours, a yellow body and a red head, the reeding between both colours with both ends also does not show any deference in results (Fig. 15).

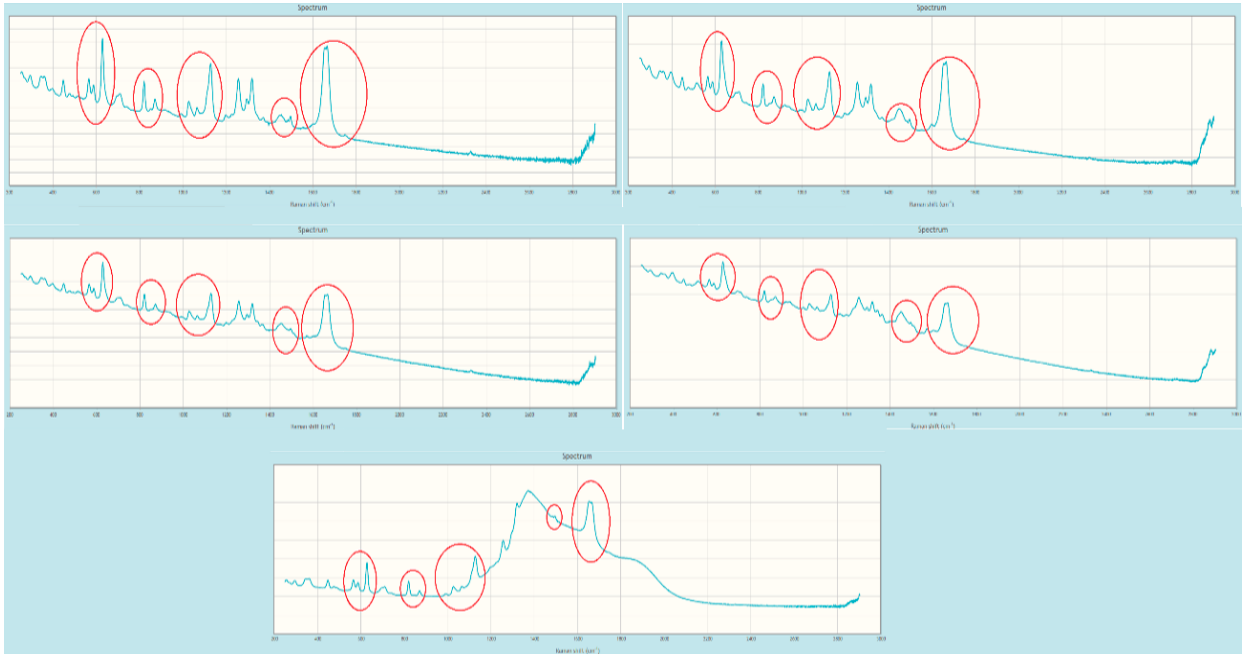

Figure 15 - Raman spectra of the component C. On top reading of yellow body (PnN to the left and PnC to the right) on full capsule, in the middle reading of red head (PnN to the left and PnC to the right) on full capsule and bellow reading of content in vial.

Once again, a slight difference in peak intensity is visible in readings of both colours but this doesn't influence the product identification.

### 6.2.4 COMPONENT D

In this product, it was possible to perform an empty capsule reading to understand if the given value was different from the content reading. This was not observed. (Fig. 16)

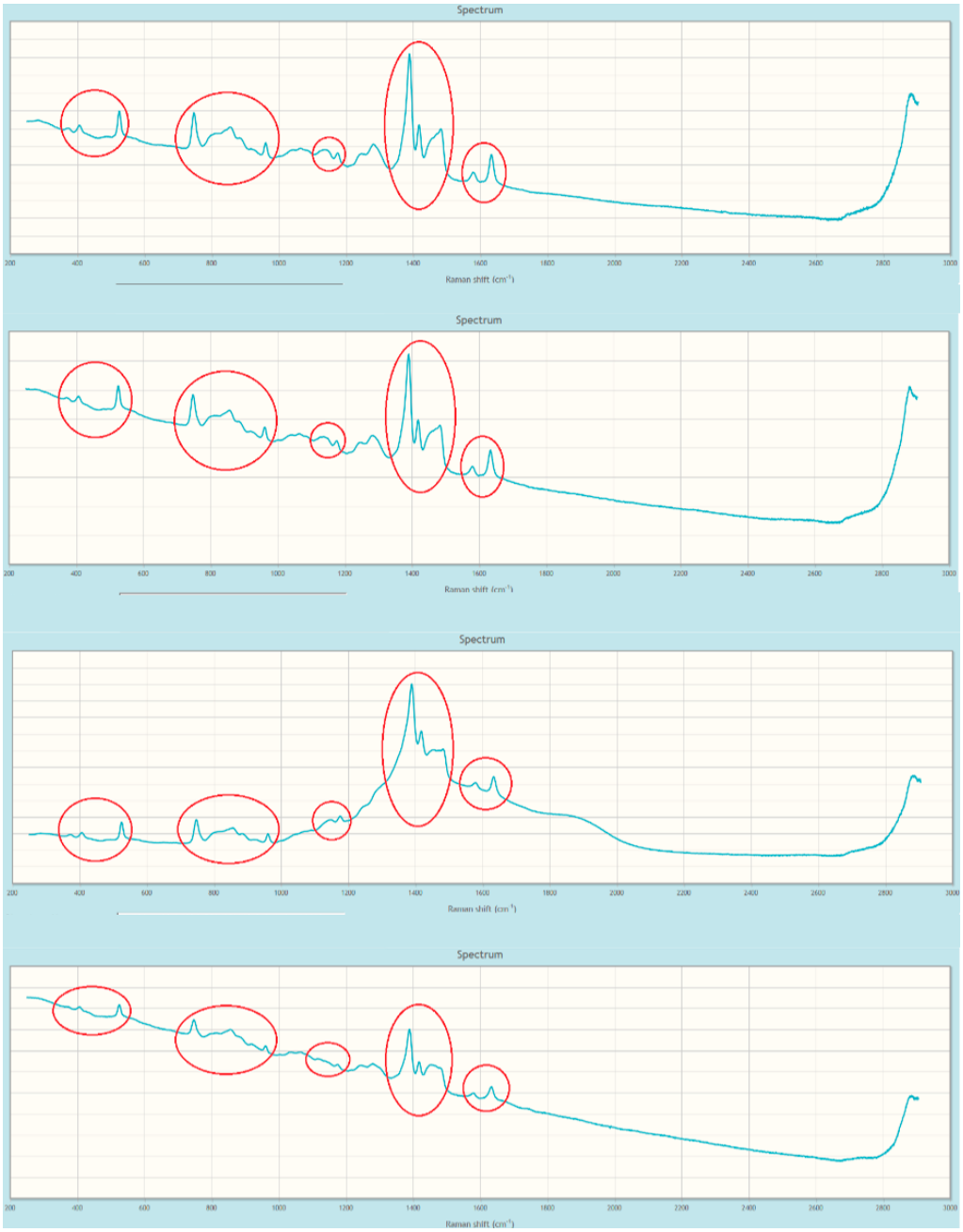


Figure 16 – Raman Spectra of component D. On top reading with PnN on full capsule, in the middle reading with PnC on full capsule and second from bottom the reading of content in vial, in the end reading of empty capsule.

Due to the capsule characteristics, washing or injecting water into the interior is not possible because of degradation. The data obtained from the empty capsule might be a result of remainings present (thin layer in the interior wall of the capsule, no accumulation and not visibly

noticeable if it is the case), where the complete extraction was not possible. Independently of reason, this didn't demonstrate any value that points to interference in the reading of the content.

### 6.2.5 COMPONENT E

Once more the results are identical to the previous being able to observe the same spikes both in the vial reading and the capsule (Fig. 17).

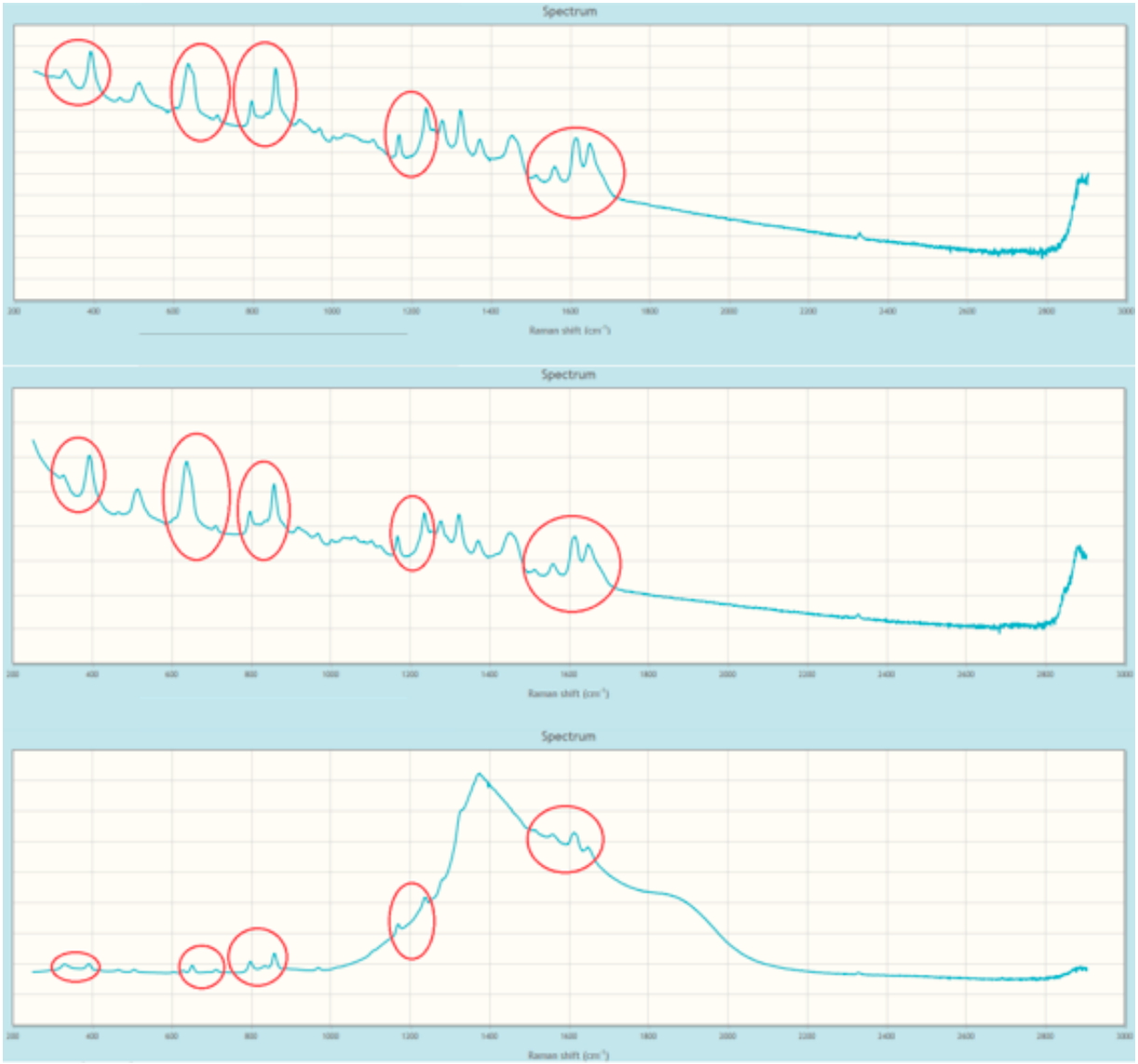


Figure 17 – Raman Spectra of Component E. On top reading with PnN of full capsule, in the middle reading with PnC of full capsule and bellow reading of content in vial.

# 7. CONCLUSION

The Raman Spectroscopy technique is seen to greatly benefit the performance of the Quality Control department by reducing the overall work required to analyse the products, being this by the reduced time spent on sampling, reduced quantity of tests required to perform, and the reduced number of samples needed to test in the laboratory.

One of the great advantages of using this technique, in addition to these increases in performance, is that its application does not require direct contact with the product. These can be analysed through the bags where they are stored, thus avoiding contamination.

These performance increases (an increase of around 65%) make the initial investment in equipment very worthwhile as it can serve as a performance enhancer and an initial test that can discard faulty products in an early control stage saving the laboratory resources and time since these do not get to be sampled.

It is not always possible to apply the technique specially to products prone to fluorescence that give off an error while reading but these are few compared to the products that benefit from its use.

The experiment performed in capsules to demonstrate if it is possible to apply the technique in these showed that it's ready to use without the need to directly manipulate the product. The readings of the content in the capsule had the same spikes regardless of reading in or out of the capsule proving that these didn't interfere with the technique's ability to acquire their content's spectra.

# 8. CONCLUSÃO

A técnica de Espectroscopia Raman trouxe um grande benefício para o desempenho no departamento de Controlo de Qualidade, reduzindo a carga de trabalho necessária para analisar os produtos, seja pela redução do tempo gasto na amostragem, redução da quantidade de testes necessários a realizar e o número reduzido de amostras necessárias para testar em laboratório.

Uma das grandes vantagens da utilização desta técnica além deste aumento de performance é da sua aplicação não necessitar de contacto direto com o produto. Este pode ser analisado através dos sacos onde estão armazenados evitando assim a contaminação dos mesmos.

Estes aumentos de desempenho (um aumento de cerca de 65%) compensam muito o investimento inicial no equipamento pois este pode alem do desempenho pode ser um lado como um teste inicial que pode descartar produtos defeituosos numa etapa inicial de controle poupando assim tempo e recursos da parte do laboratório uma vez que estes não chegam a ser amostrados.

Nem sempre é possível aplicar a técnica especialmente a produtos propícios a fluorescência que dão erro na leitura, mas estes são poucos face aos produtos que beneficiam da sua utilização.

A experiência realizada em cápsulas para demonstrar se é possível a aplicação da técnica nas mesmas mostrou que esta está pronta para o uso sem a necessidade de manipulação direta do produto. As leituras do conteúdo na cápsula tiveram os mesmos picos independentemente da leitura dentro ou fora da cápsula, provando que estes não interferiram na capacidade da técnica de adquirir os espectros de seu conteúdo.

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# Annexes

## Results Component A

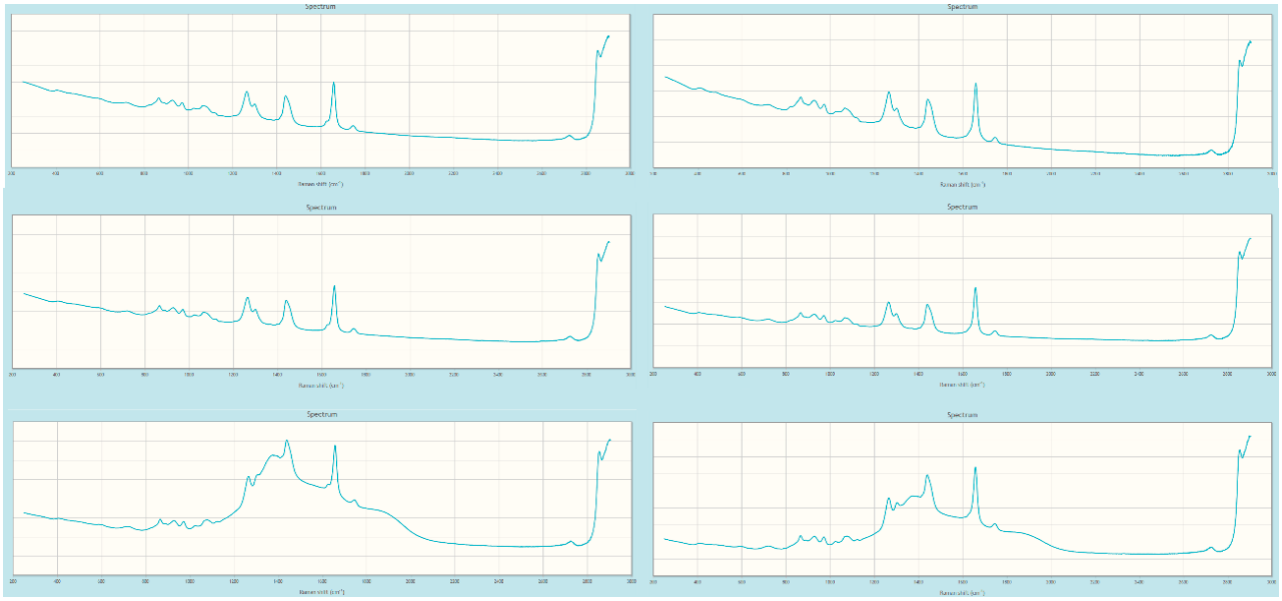

Figure 18 - Raman spectra of two different batches of component A (one two the left other to the right). Readings with PnN on full capsule on top, readings with PnC on full capsule in the middle and readings of content on vial bellow.

# Results Component B

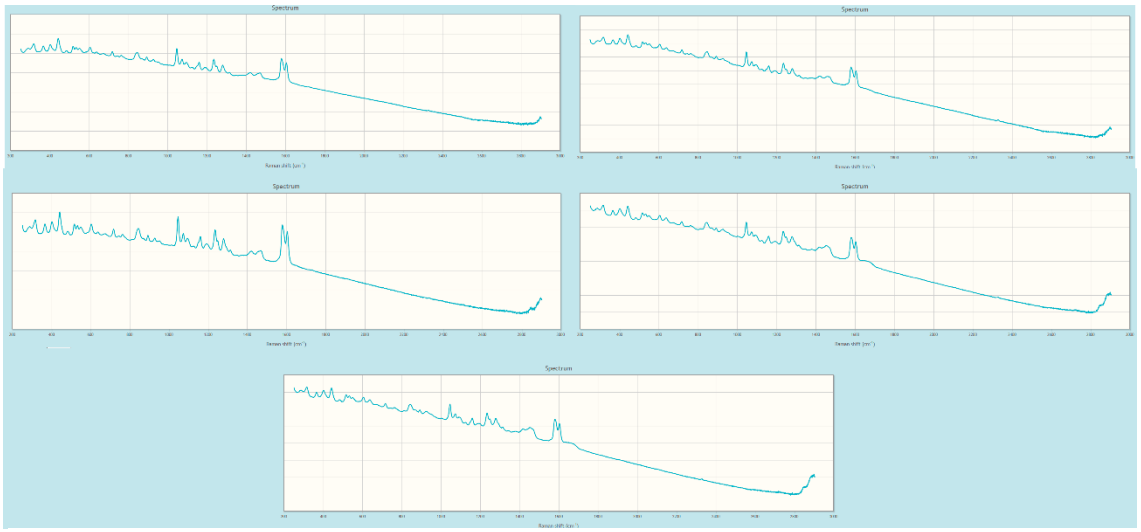

Figure 19 - Raman spectra of a different batch of component B. Reading on transparent body to the left, green head to the right. Readings with PnN on full capsule on top, with PnC on full capsule in the middle and readings of content in vial bellow.

# Results Component C

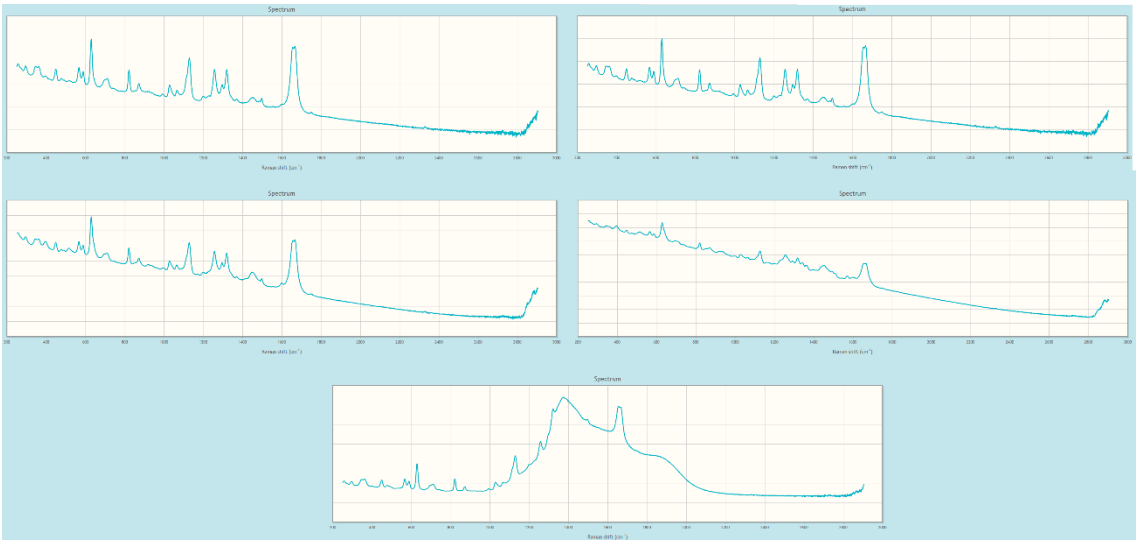

Figure 20 - Raman spectra of a different batch of component C. Yellow body to the left and red head to the right. On top readings with PnN on full capsule, readings with PnC on full capsule in the middle and readings of content in vial bellow.

# Results Component D

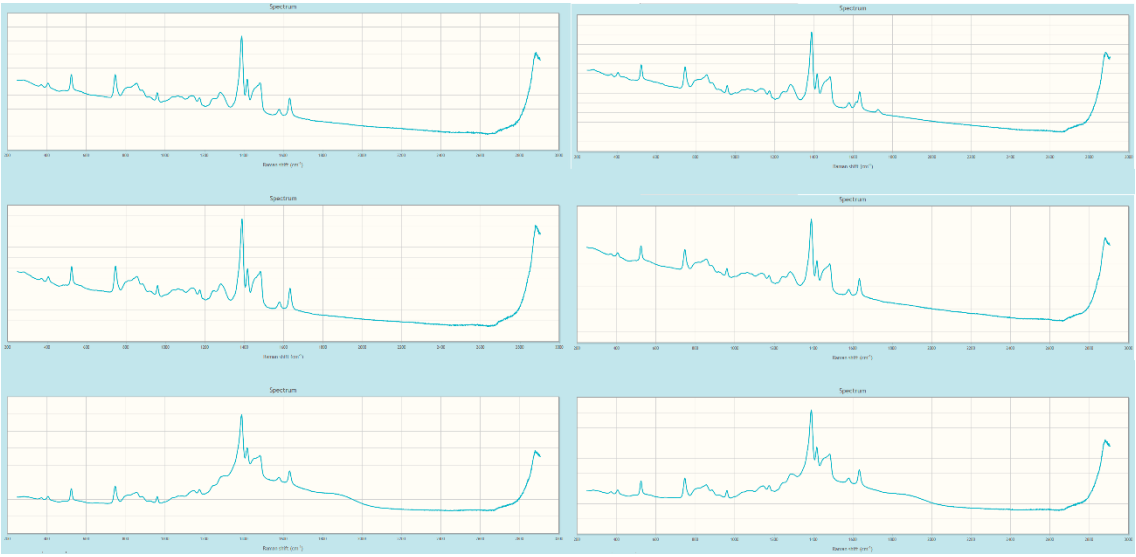

Figure 21 - Raman spectra of two different batches of component D (one to the left and the other to the right). Readings with PnN on full capsule on top, readings with PnC on full capsule in the middle readings of content in vial bellow.

# Results Component E

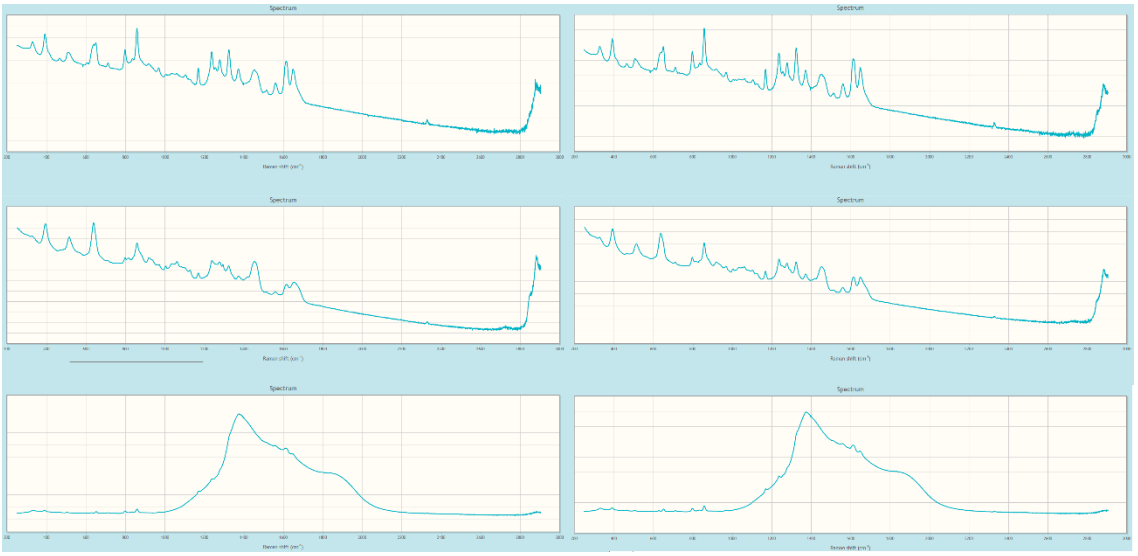

Figure 22 - Raman spectra of two different batches of component E (one left and the other right). On top readings with PnN in full capsule, in the middle readings with PnC in full capsule and readings of content in vial bellow.