An Executive Summary

Implementing Transmission Raman Spectroscopy for Fast Content Uniformity Testing: From Feasibility Evaluation to a Validated Release Method

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When considering a new analytical technology in the pharmaceutical industry, the major considerations typically revolve around two questions: Is there a good business case for it, and what are the regulatory consequences? What follows are the results of an evaluation that led to the implementation of transmission Raman spectroscopy (TRS) for content uniformity testing at Grünenthal Pharma in Germany. In the end, it was not only the financial benefit, but also the stakeholder enthusiasm that convinced management of the need to invest in new technology for a standard test requirement.

Introduction

The approach for integrating TRS to all sites at Grünenthal Pharma involved the development of procedures and templates steered by a Center of Expertise (COE). Models were developed at the COE, and the required equipment, standard approach for sample preparation, validation procedure, and regulatory strategy were also determined here. With this approach, the company not only enabled a global standard, but also ensured the transfer and support of methods from one site to another.

Transmission Raman Spectroscopy

TRS is a technique that can be applied to common analytical methods in the pharmaceutical QC lab, not only for content uniformity and assay quantitative analyses, but also for drug product identification, polymorph, and crystallinity applications. It is generally used for solid dose formulations, including tablets and capsules, but can also be used for powders and other dosage forms. Content uniformity testing with TRS can be compared to wet chemistry results such as those generated by high performance liquid chromatography (HPLC). TRS quantitative testing offers a quicker, more streamlined process for releasing products, with a low cost per test and virtually no consumables. Agilent exclusively offers both HPLC and TRS technologies to best fit the needs of a QC lab.

HPLC and TRS are complimentary analytical techniques. Where HPLC can be used for impurities, degradation products, and dissolution testing, TRS is a great fit for solid-state properties as samples are analyzed intact. Non-destructive testing means polymorph and amorphous API quantitation, together with residual crystallinity, are excellent applications for TRS. Content uniformity testing is an area of major benefit for the TRS technique because for higher volume testing, HPLC analysis comes with a high resource burden within a lab. Formulation development is also a good fit for TRS analysis when there is a demand for high throughput testing or a change of formulation. TRS can easily be utilized at-line in a production environment where spectroscopy is more commonly used.

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The workflow differences between TRS (TRS100, Agilent Technologies) and HPLC are exemplified in **Figure 1**. A major advantage of TRS analysis is the minimal sample preparation time compared to HPLC analysis, which requires significant preparation steps and a suitable calibration standard. This process is manual and labor intensive, making it a potential source of error and additional cost. TRS simply requires a tray to be loaded with samples, requiring little skill or analyst touch-time. TRS is also highly flexible. In order to change between product methods, a new tray is loaded and a different method selected, meaning many different methods/products can be run, one after another on the same instrument, with very little downtime in between.

TRS has several advantages for the analysis of pharmaceutical samples including:

- Non-destructive in nature
- Simple workflow
- No sample preparation
- No solvents used
- No waste to dispose of
- No consumables

The overall benefit means that testing can move from a QC lab to the production line, where release tests or process validation can be carried out right next to the tablet press or capsule machine. The TRS100 is 21 CFR Part 11 compliant as well as meets the strict requirements of *United States Pharmacopeia* (*USP*) Chapter <1120> and *European Pharmacopeia (Ph.Eur)* Chapter 2.2.48 for Raman spectroscopy.

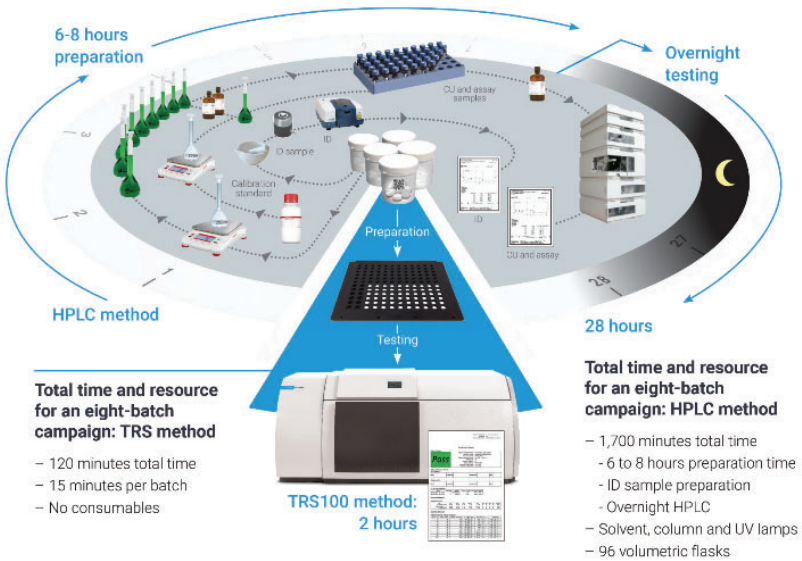
Principles of TRS

The fundamental principles of TRS are shown in **Figure 2.** TRS uses a large spot laser beam to illuminate one side of a tablet or a capsule. The light scatters through the sample and is detected on the other side, resulting in a Raman spectrum. The benefit of this approach over conventional Raman is that the spectrum is representative of the whole sample, not just surface sub-sampling. TRS also works through capsule shells and tablet coatings, so no sample preparation is needed.

The TRS100 system is fully automated. Capsules, tablets, and powders are loaded onto a tray and the X-Y stage inside the instrument then moves each material into the sample beam, with samples analyzed sequentially. Each sample typically takes 10–20 seconds to analyze so a content uniformity test of 10 tablets only takes a few minutes. This makes TRS an ideal solution for high volume testing of many batches per year; the more batches that require testing, the more beneficial the technique becomes. The TRS technique works with various types of samples and presentation methods, including powders in zippered plastic bags, well plates, and oral solid dose forms.

A comparison between TRS spectra and a HPLC chromatogram is observed in **Figure 3**. In the HPLC chromatogram, the separation of the peaks is a function of the elution time, which is typically 10–15 minutes, depending on the detector and column material. In a TRS measurement, the entire signal is collected as the measurement takes place, generating an information-rich spectrum representing the different components, including excipients and APIs. The complexity of the spectrum means that multivariate techniques are necessary to extract information from the data,

Figure 1: Comparison of analytical workflows between the TRS100 and HPLC for content uniformity.



TRS100 speed

- No sample preparation
- Takes <15 minutes to run a batch

Costs

- Avoids consumables/solvents/waste
- Takes little user skill or touch time

Flexibility

- Change methods in seconds
- Tests can be completed as needed, without scheduling

and this is how quantitative information is produced. The spectrum in Figure 3 was captured in one second, but longer measurement times can be used for increased sensitivity.

HPLC is a primary reference technique, so generally when the release method is submitted to a regulatory agency, TRS is presented as a secondary reference technique giving equivalent values to the HPLC method. The HPLC technique relies on a reference standard detector response (based on concentration) with sample measurements compared to the standard to generate a quantitative result. In contrast, when using TRS methods, a fixed calibration is developed by showing all concentration variances in advance. A prediction model is created, which is a function of the variances of the API and excipient concentrations. An experiment is then

Figure 2: Principles of transmission Raman spectroscopy.

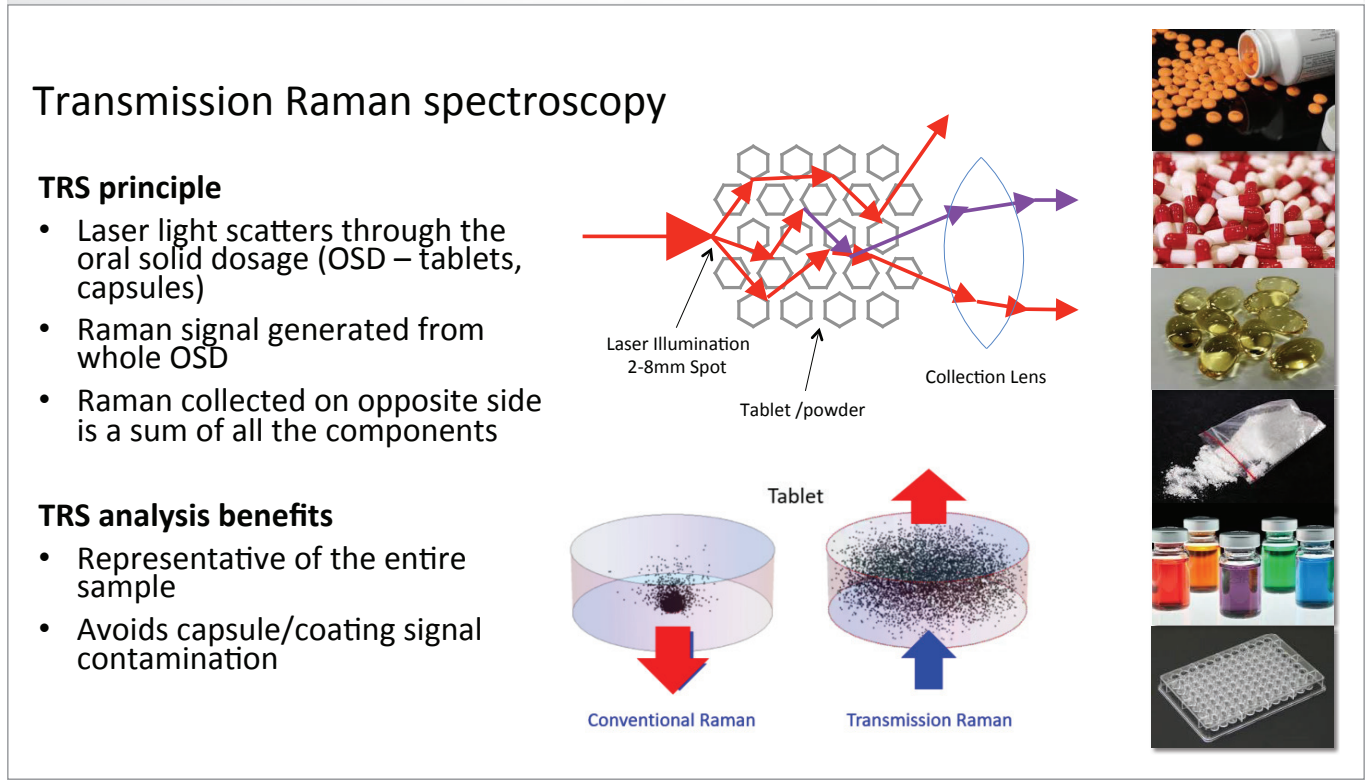
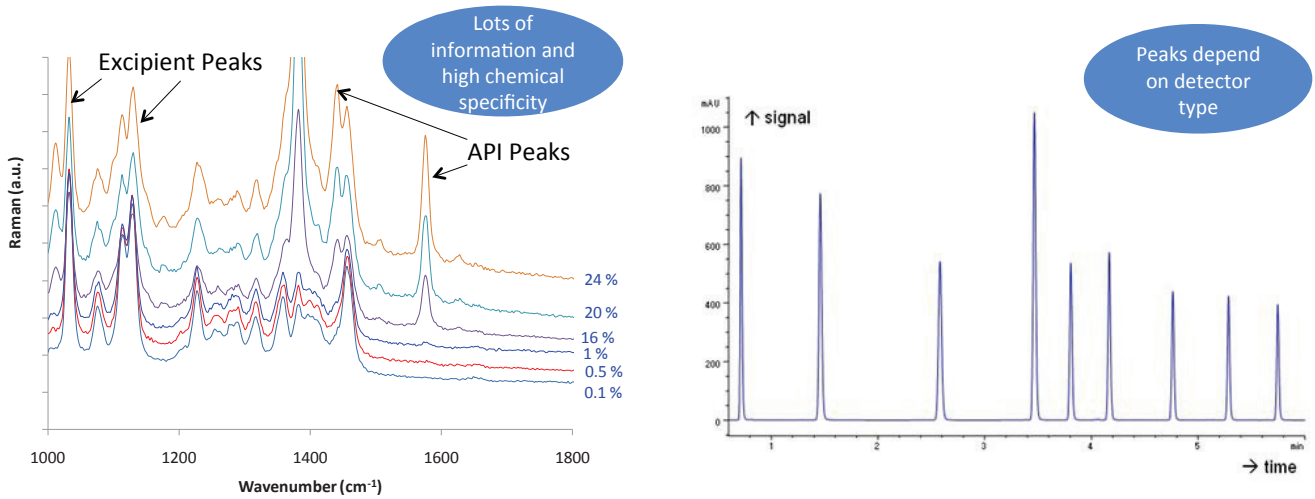


Figure 3: Comparing TRS spectra to a HPLC chromatogram.



TRS spectra captured in **1 second** on a TRS100
Single spectrum of the entire sample

HPLC Chromatogram takes minutes
Peaks separated by elution time

designed by building a model of an output spectrum where the API and excipients change as a function of concentration. The benefit is that once a method is developed, there is no need to run a reference standard again.

TRS at Grünenthal Pharma

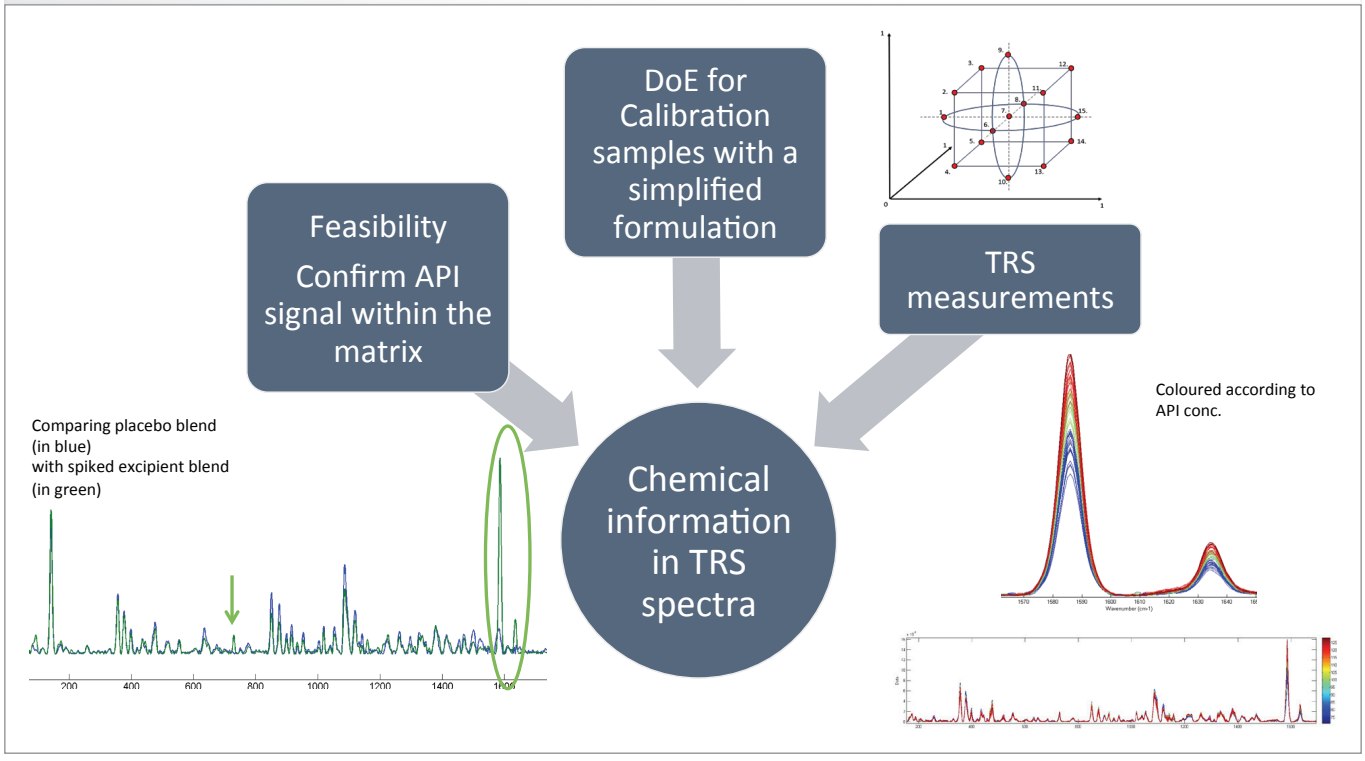
Grünenthal had been assessing the possibilities of applying the TRS100 to pharmaceutical analysis—from feasibility to validation—for the past two years. The company had previously evaluated spectroscopic process analyzers to facilitate parametric release, which has an enormous regulatory cost. Since none of the cases had been truly successful, Grünenthal management was extremely skeptical of anything related to a solution that used molecular spectroscopy techniques.

Nevertheless, Grünenthal Pharma researchers tested the potential of applying spectroscopic methods in quality control using a near infrared (NIR) spectrometer and Raman spectroscopy in the reflectance mode. They also investigated whether it was possible to do batch release testing of tablets and coated tablets using TRS. The focus was set on batch release testing of content uniformity, with already approved applications for products of other companies at the EMA and the FDA, plus the related assay testing.

A study was set up to test a variety of tablets with low to high API contents, plus tablets containing two APIs. These tablets were tested with NIR, reflectance Raman, and TRS techniques. The results clearly showed the superiority of Raman over NIR. For NIR, with sufficient data preprocessing, it was possible to get "calibration" models for one of the three products, but the error was high and not comparable with HPLC. The main difficulty here was to get differentiable signals from the API (for the low concentrated APIs) within the tablet matrix using a NIR diffuse reflectance probe. However, both Raman systems gave good results. The TRS100 was superior for one product, which showed only a distant API peak below 150 wavenumbers (the so-called Phonon region), a range that was not covered by the other Raman spectrometer. So, the technical feasibility was demonstrated through all products except for one fluorescent API of 0.04% coating, where the TRS100 was unable to get any kind of signal from the matrix. Fluorescence is an effect that can swamp a weak Raman signal and thus the combination of a low concentration with an API emitting fluorescence is the worst-case scenario. Additionally, the TRS showed a higher degree of usability, because it had a tray instead of presenting the tablets one by one to the Raman probe, providing benefits in terms of efficiency, process robustness, and human error. All these factors then became the basis of a business case justification for the TRS100.

The advantages of the TRS technique over HPLC are obvious, as samples do not need to be diluted, spiked, or destroyed. The drawback in establishing a spectroscopic method like this is that one needs to apply multivariate data analysis. This aspect can be very difficult to explain, and also to understand, and it makes it difficult to increase the support for the methodology. Knowing this, the group organized crash courses on multivariate data analysis and Raman spectroscopy to increase the level of understanding and consequently

Figure 4: TRS100 feasibility study and DoE for calibration - Focusing on what is known.



the support, enabling colleagues to understand the difference between a good and a bad model. Additionally, the team focused on similarities between HPLC and Raman when presenting the method.

Feasibility Study

Evaluation began with a feasibility study confirming that concentration-dependent API signal changes were detectable within the formulation matrix. The next step was to carry out a Design of Experiment (DOE) for the calibration by varying all major components between 70% and 130%. The choice of data preprocessing was visually confirmed by color-coding the spectra according to respective API concentration. This is shown in **Figure 4**, where the blue peak is equivalent to 70%, green is 100%, and red is 130%. Measuring a Raman spectrum of a tablet does not only enable the quantification of the API but also of the excipients. Thus, the spectra can also be color coded according to minor components in the blend, if required.

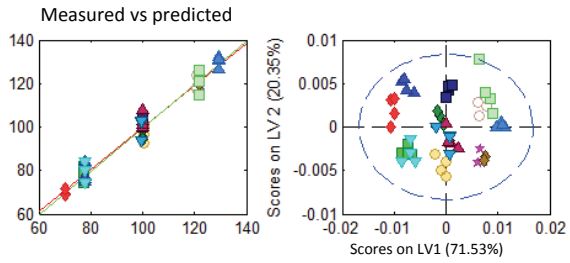
Based on chemically validated preprocessing (identifying a suitable preprocessing using color coding), a partial least squares (PLS) model was developed. One should consider the background of the audience when presenting the results: Calibration curves are widely known and well understood, thus they can be presented to nearly any audience. Scores plots require a solid understanding of principal component analysis (PCA). In the case of a calibration DoE it can also support the understanding by showing the underlying structure of the DoE cube being represented in the scores plot, as demonstrated in **Figure 5**.

Capability of TRS Compared to HPLC

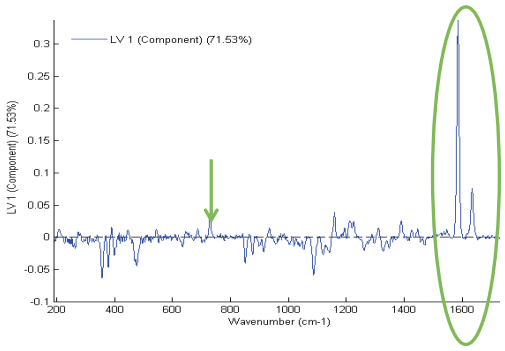
Although it might be difficult to explain what the Raman method actually does, it is much easier to relate the differences in the chemical information in the sample by showing the variation in the latent variables. **Table 1** is an overview of the comparison between TRS and HPLC for a tablet matrix. With Raman, the sample is simply placed inside the instrument and within a few seconds the multivariate spectral information is generated, whereas with HPLC, quite lengthy sample preparation is needed before the separation process. With Raman spectroscopy, data preprocessing is performed to focus primarily on chemical information and minimizing the effect of physical interactions, while HPLC uses a column and separates the different sample components by polar interactions between a mobile and stationary phase. Raman uses scores plots and latent variables to reduce sample dimensionality. The scores present variations in data and latent variables give feedback to the wavelength information, whereas HPLC carries out peak detection, integration and measurement.

For Raman, one typically calibrates using a partial least squares regression, while for HPLC a simple univariate linear regression is applied. The measurement terminology is a little different for both techniques. For Raman, it is a “prediction

Figure 5: TRS100 results of a calibration DoE - Calibration curves, scores plots and latent variables.



- Calibration curves are known, thus worth presenting
- Scores plot – already difficult to explain but obtains important information
  - Samples are grouped according to their chemical information (looks like DoE cube in 2-D)
  - First two latent variables explains here 91.88% of spectral variation and helps to train people in differentiating between a good and bad model



Latent variables

- Positive peaks are positively correlated to an increase in API conc.
- Show the latent variables and make the link to the chemical information – that gives confidence

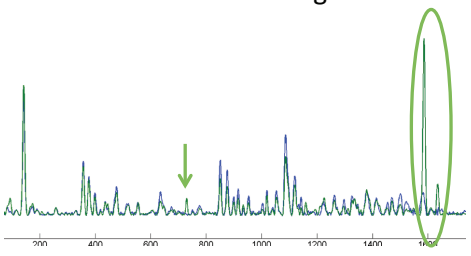
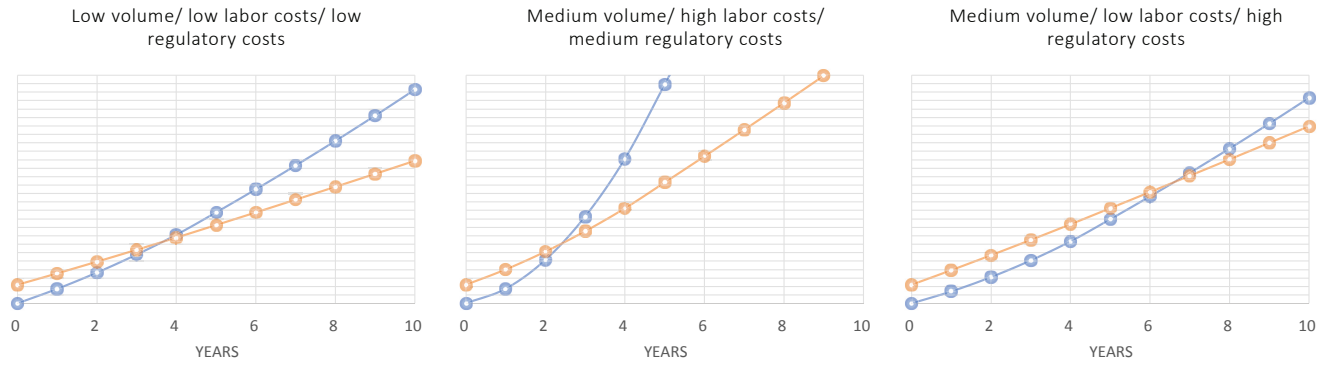


Figure 6: A comparison of volume/labor/regulatory costs of HPLC and TRS together with payback times.



- Possible to calculate a BC for a TRS100 with a reasonable payback even with low volume products (e.g. 50 batches per year)
- Greatest drivers:
  - Negatively: Regulatory costs, low labor costs
  - Positively: high number of batches, high labor costs and low optimization status of HPLC methods
- HPLC costs should not be neglected in the costs for TRS methods since they are still required for validation and method maintenance

of concentration of X", whereas for HPLC, it is a "measurement of concentration of X" where X is the analyte of interest such as excipient or API. Even though their descriptions are slightly different, they are actually very similar because they both use regression curves.

Table 1: Comparison of Raman and HPLC for a tablet matrix.

	Raman		HPLC
Tablet Matrix	Multivariate spectral information	Sample preparation is not required	Sample preparation
Separation of information	Data pre-processing	Information is separated after measurement	Via HPLC Column
Next step	Scores – variation in data; Latent variables - Wavelength	Reduce dimensions: 2x 2 dimensions	Peak detection and integration
Calibration	Partial least squares regression; Link to e.g. chemical information		Linear Regression
How do we call it?	Prediction of API conc		Measurement of API conc

…. Not so different, is it?

Business Justification

The business case justification was not easy because HPLC is the industry standard and the requirements for method validation and method development are well documented in the public domain, whereas TRS is a new technique that is just being applied to the demands of the pharmaceutical industry.

Depending on the efficiency and volumes, five to eight HPLC systems can be realistically replaced by one TRS100. However, to make a valid justification the group decided to base the comparison on five of them, based mainly on sample throughput and time required for analysis. Additionally, it makes the business case a little more difficult when you only have one Raman system and you cannot completely eliminate HPLC because validation work, method development and model maintenance need to be carried out. In addition, the measurement of impurities still has to be done by HPLC because Raman is not really suited to this task. Another factor to consider is that when investing in Raman for the very first time, additional training time is required because it is an entirely new system.

A more detailed breakdown of costs assumptions are tabulated in **Tables 2 and 3.**

Applying the Business Case

Figure 6 describes several scenarios using different assumptions. One example is based on low volume batches, low labor costs, and also low regulatory costs, resulting in a payback time of approximately four years. The greatest drivers on the negative side were the higher regulatory costs and low labor costs/low volumes and on the positive side, the higher

Table 2: The business case for TRS100.

	HPLC	TRS100
Pieces of equipment	5-8 Depending on time required for analysis (average)	1 Raman + ½ HPLC (for validation/ revalidation and model maintenance)
Investment	Equipment: Already available but renewal of required HPLCs	Equipment and training required Renewal of 0.5 HPLC for validation etc
Method development and validation	Only HPLC	HPLC + TRS100 method + costs for regulatory change (average costs x countries)
Routine analysis costs	Maintenance costs x pieces of equipment Costs per 100 batches incl. men hours, filters, columns, reagents/solvents and standards	Maintenance costs + 10% of HPLC maintenance costs Costs per 100 batches incl. men hours + 10% of the costs for HLPC routine for revalidation
Depreciation	10 years x number of pieces of equipment	10 years 1 TRS100 + ½ HPLC

Table 3: Routine analysis and savings.

Routine analysis	HPLC	TRS100
Preparation of standards and samples	x	few minutes
Preparation of Equipment	x	few minutes
Runtime	x	<20min
Integration	x	0
Review of results	x	5
Total time until result [min]	**~300**	**~30**
Required labor time [min]	**>150***	**~20**

Highly dependent on individual analysis and degree of optimization. (e.g. HPLC campaign)

the number of batches the better the justification. It is very important to be aware that the HPLC costs should not be neglected for Raman methods because they are still required for validation and model maintenance.

A scenario with medium volume products, high labor costs, and medium regulatory costs results in a payback period of about 2.5 years. For production, it is relatively easy to calculate cost reduction because the prices and the cost of analysis are transparent. However, the complexity of the financial model depends on the degree of detail used to calculate the savings. It is reasonable for the first estimation to calculate the average time and costs required for HPLC, and also an average of the regulatory costs per country. The downside is if the products are already on the market, as the regulatory cost may have a significant impact and thus require a suitable regulatory strategy. A TRS100 system can also analyze physical-chemical information like crystallinity, amorphous state, solvate, polymorphic forms, and other physical information such as size distribution and tablet hardness so there is a significant upside for galenic development and transfer, for instance, which is difficult to put in numbers. In addition, one can get chemical information on the API and all excipients that have been calibrated.

For products already on the market, a two-pronged approach was used: In-Process Control (IPC) testing, without any regulatory impact, and for products that had a high volume but a limited number of registrations. For example, one can define a regulatory strategy if 90% of a product is sold in one geographical region such as the European Union (EU), requiring only a Type 1b change. So, the group decided to develop a strategy to have two material numbers for the same product; two batches for all other markets outside the EU and the rest of the batches produced for the EU market, where they expect a relatively quick regulatory approval.

Additionally, there is a positive impact for formulation development. A Raman model can be created within a day or two and used for comparative analysis of assay during formulation development. Samples can then be analyzed within minutes instead of days or months for HPLC. They saw a benefit due to the recent approach of applying quality by design to any step in formulation development. This usually means that a process DOE must be carried out, which requires large numbers of samples to be measured. In the case of a Raman model, it helps to get faster access to analytical results, and also to get an in-depth knowledge and understanding of the process. So, as a result, they determined an interdisciplinary approach would be best since it would allow them to exploit the full potential for cost saving.

Summary

The final part of the technical feasibility study was to implement a strategy to fully test the system on all their products, including tablets, capsules, powders, granules and sieve fractions. The results have been very encouraging, with only products containing extremely low levels of API (<0.04%) posing a slight problem. The future priorities will include IPC sets without regulatory impact, products to be registered and products on the market with high volume and limited number of registrations. They will continue with their evaluation and are currently setting-up global guidelines for method and model development. They are also searching for additional technology to speed up sample preparation, including an automated powder dispenser, suitable blender for 2–5 gram blends, and a tablet press for miniature batch sizes.

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