Identity Testing of Raw Materials using Raman Spectroscopy: Precursor and API related Substances

Relevant for: Pharma

Cora 5001 together with the pharma compliant Spectroscopy Suite desktop software is a fast tool for identity testing of precursor and API related substances. Method development and validation according to USP regulation is easily possible.

The pharmaceutical production industry is one the most heavily regulated markets in the world. From the delivery of raw materials to the packaging of final products, the production processes need to be monitored according to legal requirements ensuring product quality and minimizing risks for the patients in all parts of the world. The Active Pharmaceutical Ingredients (APIs), the pharmacologically active part of a drug product, need to be tested among other things for their chemical identity as stated by international pharmacopeias and GMP guidelines.[1], [2], [3] In addition, drug formulations may include precursors or API related substances such as degradation products if there were e.g. problems during production procedure or storage.

Here, fast and accurate methods are required to quickly assess whether a batch is fit for further use or needs to be rejected. Anton Paar's Cora 5001 Raman spectrometers together with the Spectroscopy Suite desktop software offer fast and reliable ID testing using algorithmic database matching. Method development and validation are straightforward and processes are easy to handle due to the intuitive user interface. As the software is fully compliant to CFR 21 part 11 according to FDA, data integrity and a complete audit trail are ensured.[4]

1 Active Pharmaceutical Ingredients (APIs)

According to GMP an active pharmaceutical ingredient (API) or drug substance is "any substance or mixture of substances intended to be used in the manufacture of a drug (medicinal) product and that, when used in the production of a drug, becomes an active ingredient of the drug product".[5] Furthermore, GMP states that these "substances are intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure and function of the body."[5]

According to international pharmacopeias identity testing is required for every API used in drug production as stated in the respective chapters.[6], [7]

2 Requirements for ID Testing with Raman Spectroscopy according to USP

According to US Pharmacopeia chapter 197, Raman spectroscopy may be used for identity testing of raw materials such as APIs.[7] The following recommendations apply: measurement device should be prepared for the measurement according to the manufacturer's recommendations and a performance check should be conducted prior to use. Spectra of sample and references should be collected using the same instrumental parameters. The resulting spectra are then compared qualitatively. Where available a USP reference standard should be used as reference sample.[6] The qualitative comparison can be conducted algorithmically using chemometric models.[8] The method has to be validated according to USP 1225.[9] For qualitative analyses the specificity and the robustness of the method have to be demonstrated during method validation.[9], [10]

3 ID Testing using Raman Spectroscopy

3.1 Experimental Setup

For ID Testing of the APIs, precursors and API related substances a Cora 5001 Direct Raman spectrometer with an excitation wavelength of 785 nm was used (see Figure 1). The instrument is compliant to USP 858 and therefore perfectly suited for ID testing using Raman spectroscopy.[10] It was controlled via Anton Paar's Spectroscopy Suite desktop software which fully complies to CFR 21 part 11 according to FDA, ensuring data integrity.[4]

3.2 Method Development

The instrument was checked for correct calibration with the supplied System Suitability Test (SST) standard prior to use. As a first step to method development, test runs for optimizations of measurement conditions were conducted. The implemented autofocus and automatically determined exposure time render this process swift and hassle-free. The obtained parameters were saved in a method which was submitted, reviewed and approved by the responsible users. Subsequently, a good quality library spectrum of each reference substance was measured using the previously developed method and submitted for approval. After approval by the responsible lab manager the spectra were fit to be used in a spectral library.

3.2.1 Spectral Library Development, Publication and Approval

For each substance a library entry with the corresponding spectrum was created. The software offers the possibility to enter additional information on the substance such as further names or GHS symbols as well as a comment field for other relevant information (e.g. CAS number, purity, supplier). Subsequently, each substance entry and the whole library are submitted for approval. After approval the library is fit for use in an ID testing method.

3.2.2 ID Testing

On another day an identification method was created using the same parameters as used for the measurements of the library spectra, as required by USP. The software offers to copy the method used to acquire the reference data and to add identification parameters for the intended analysis, therefore equality of the measurement parameters can be easily achieved. The new method was signed (submit, review, approve) as well by the responsible employees. Subsequently, samples of API, precursors and API related substances (see Table 1) were measured and identified.

Table 1: List of measured APIs and their precursors.

API	Sample group	abbreviation
Acetylsalicylic acid	Analgesic	API 1
Salicylic acid	precursor of API 1	P1.1
Acetic Anhydride	precursor of API 1	P1.2
Ciprofloxacin	antibiotic	API 2
2,4,5-Trifluoro benzoyl chloride	Precursor of API 2	P2
Ibuprofen	Non-steroidal anti-inflammatory drug	API 3
Isobutyl benzene	Precursor of API 3	P3
Clarithromycin	antibiotic	API 4
Erythromycin	Precursor of API 4	P4
Acetaminophen	Analgesic	API 5
4-Aminophenol	Precursor of API 5	P5

3.3 HQI Matching Algorithm

For the comparison of reference and sample spectra the Anton Paar Spectroscopy Suite offers an HQI matching algorithm. The results of the algorithmic matching lie between zero for no correspondence and 100 for identical spectra.

By defining a suitable HQI threshold the identification or verification of substances can easily be achieved.

4 Results and Method Validation

Table 2 shows the resulting matches of each sample against each reference spectrum using the HQI (Hit Quality Index) matching algorithm. It is clearly visible that no substance is mismatched as the respective API using an HQI threshold of 75 or higher. The specificity of the method is clearly visible when comparing the spectra of structurally very similar substances such as the sample group of the acetylsalicylic acid (API 1) and its related substances salicylic acid (P1.1) and acetic anhydride (P1.2). As an HQI of 100 corresponds to a perfect match meaning identical spectra, there is no risk of false identification of one of the precursors as the actual API as the salicylic acid with the closest

Table 2: HQI Matrix showing specificity and robustness (average of triple measurements) of ID testing method.

		Test samples (average of 3 measurements)					
		P1.1	P1.2	P2	P3	P4	P5
Reference substances	API 1	**8.52**	**0.44**	2.56	1.64	0.26	4.29
	API 2	2.22	0.13	**3.71**	0.56	2.05	4.57
	API 3	0.26	0.01	3.19	**13.05**	18.09	8.33
	API 4	2.50	0.02	1.61	1.62	**73.75**	0.12
	API 5	15.00	0.13	3.51	0.24	1.30	**14.30**

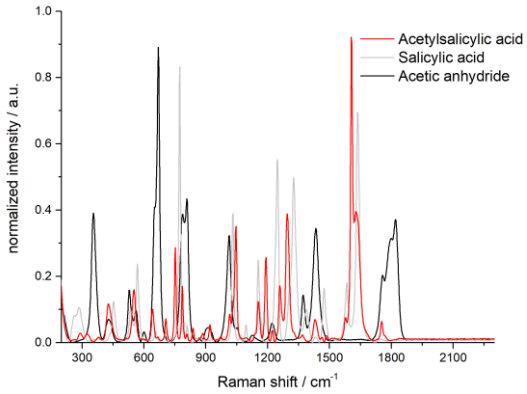

Figure 1: Raman spectra of Acetylsalicylic acid (API 1) and its precursors. Spectra have been normalized to the highest peak.

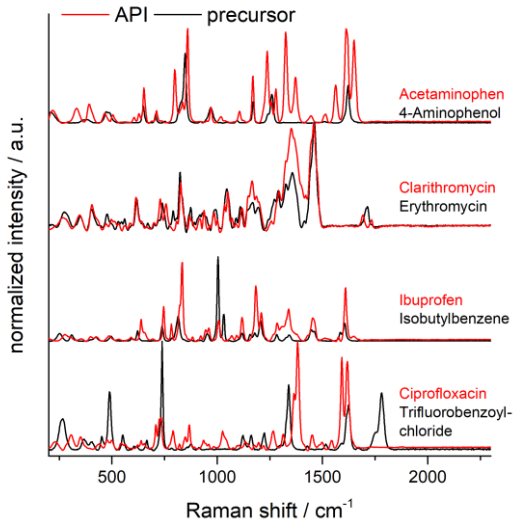

Figure 2: Raman spectra of APIs 2-5) and the respective precursors. Spectra have been normalized to the highest peak.

correlation to the API with HQI 8.52 differs by more than 90 from a match as acetylsalicylic acid. Therefore, the substances are well separable with the implemented algorithm though they are chemically closely related (see Fig. 2).

Despite chemically close relation of clarithromycin and erythromycin, these substances are also well separable using an HQI algorithm as using a threshold of 75 or higher will avoid mismatches. The visual comparison of the different spectra as shown in Fig. 3 again illustrates the discrimination power of Raman spectroscopy for the different mycins as well as for the other tested APIs. The HQI results of API and the respective precursor differ by more than 80 for acetaminophen, ciprofloxacin, and ibuprofen again, showing an excellent discrimination using the simple HQI algorithm.

Measurements were performed in triplicate to also evaluate repeatability, standard deviations varied between 0.001 to 0.2044 of the HQI within the respective pairs. It is recommended to validate a method by measuring spectra on different days and by different instrument operators. After validation the method is fit for use in the production.

5 Conclusion

Raman spectroscopy using Anton Paar's Cora 5001 Raman spectrometer together with the Spectroscopy Suite Desktop Software speeds up identity testing for APIs and their related substances such as precursors or degradation products. Validation of the method demonstrates specificity and robustness of the procedure and is established equally fast as the test measurements themselves.

6 References

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