USP Technology Review: CBEx

This report is one of an ongoing series of reports evaluating the capabilities of various screening technologies, performed under USP's established Technology Review program (see Introduction for details).

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Executive Summary

A technology review was carried out on the CBEx, a handheld Raman spectrometer. The objective of the review was to determine whether CBEx can feasibly be used as a first-line screening technology to identify the presence of active pharmaceutical ingredients (APIs) in select drug products. The performance evaluation involved the analysis of three coformulated tablet samples (artemether + lumefantrine, rifampicin + isoniazid + pyrazinamide + ethambutol, and rifampicin + isoniazid), two single API and one coformulated capsule samples (amoxicillin, acetaminophen, and acetaminophen + aspirin + caffeine), and two injection samples (oxytocin and furosemide). Samples were analyzed through their packaging as well as directly against the dosage form, both as is and powdered. Samples of different dosage strengths were used, and to mimic substandard or falsified medicines, some samples were degraded by heat and humidity exposure. The instrument was able to reliably identify several of the APIs in the various drug products (acetaminophen, amoxicillin, lumefantrine, pyrazinamide) and could effectively distinguish between degraded and non-degraded samples for all of the solid oral dosage forms tested. However, it encountered challenges identifying the presence of multiple APIs in some of the coformulated products, specifically artemether + lumefantrine tablets, rifampicin + isoniazid tablets, and rifampicin + isoniazid + pyrazinamide + ethambutol tablets. It was also not able to detect the presence of API in either of the two injection samples. The field evaluation showed that most inspectors, chemists, microbiologists, and pharmacists with various levels of technical experience from the regulatory authorities of two countries, India and Zimbabwe, could become either basic, intermediate, or advanced users of the technology in less than two weeks. CBEx functioned well in the field, running off two AA batteries and collecting data quickly and presenting results simply. Although like all Raman spectrometers, the instrument has limitations related to the analysis of fixed dose combination and low concentration products, particularly injections, overall it could effectively identify the presence of APIs in a variety of solid oral dosage forms.

Recommended Citation

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1. Introduction

Assuring the quality of medicines along all points of the supply chain is vital for promoting positive health outcomes for patients around the world [1]. The importance of medicine quality screening technologies as part of this endeavor is becoming increasingly recognized [2]. USP has launched the Technology Review program, an initiative guided by a technical expert panel established through the organization's collaborative and volunteer-driven governance. The Technology Review program works towards four objectives:

1. Develop standards and guidelines for evaluating medicine quality screening technologies.
2. Generate and disseminate tailored information on the capabilities of these technologies through a two-step review process; a lab-based technical performance evaluation and a collaborative field-based utility evaluation.
3. Build the knowledge of key stakeholders to appropriately procure and sustainably utilize screening technologies for the purposes of combating substandard and falsified medicines.
4. Foster the development and enhancement of new and emerging screening technologies.

This report contributes directly to objectives two, three and four and is the second in what will become an ongoing series evaluating the capabilities of various promising screening technologies.

Advances in Raman spectroscopy over the last decade have led to the development and commercialization of an increasing number of handheld Raman spectrometers, some of which are used in low- and middle-income countries (LMICs) to screen suspicious medicines [2]. Raman spectrometers generate spectral signatures of products, which can be compared against a known sample or standard to identify whether or not the purported active pharmaceutical ingredient (API) is present. Metrohm Raman's CBEx[1] is one of the new handheld Raman spectrometers on the market. It is smaller in size and significantly cheaper than most of the other commercially available instruments. With input from the expert panel and other stakeholders, the program decided to review CBEx.

[1] CBEx was bought from Ocean Optics by Metrohm Raman in July 2017. There have been no hardware or software changes made to the instrument but it was formerly called in the ID Raman mini.

2. Methodology

2.1. General Information

Table 1 provides general information on CBEx: namely how it functions, its basic specifications, and the upfront and recurring costs of using the instrument. All data in this section were collected through email exchange, telephone conversations, and review of the vendor's website between April 2017 and August 2017.

The CBEx was formerly called the ID Raman mini. This product line was bought from Ocean Optics by Metrohm Raman in July 2017. There have been no hardware or software changes made to the instrument.

Table 1: General Information

Technology	CBEx is a handheld Raman spectrometer. The instrument comes with a built in vial holder and a point and shoot adapter. A Surface Enhanced Raman Spectroscopy (SERS) adapter is available for purchase. The instrument uses a laser to generate a spectrum of Raman active materials in a sample.
Specifications	*Dimensions:* 9 cm (H) x 7 cm (W) x 3.75 (D) *Weight:* 335 grams *Power source:* 2 AA batteries or a micro-USB cable connected to a computer *Laser wavelength:* 785 nm (1064 nm option available) class 3B laser *Spectral range:* 400 – 2300 cm^{-1}
Relative Cost	*Upfront cost* • 1 unit: $16,000 USD[2] • SERS adapter: $349.35 USD • Pharmaceutical spectral library: $2,400.00 USD *Recurring costs* • Vials: $30 (pack of 144) • SERS Gold (Au) substrates (pack of 5): $64.00 USD • SERS Silver (Ag) substrates (pack of 5): $68.00 USD • Calibration standard: $100 *Approximate cost per test (not including cost of sample)* • $0.10 USD

The cost per test is based on the calculation described below:

> The only component of the instrument with a lifetime is the laser, which has a lifetime specification of 10,000 working hours. It conservatively takes approximately 20 minutes to develop a library of spectra for one sample to use as a comparator. Once a library has been created it takes approximately two minutes to analyze a sample, which means 20 samples can be analyzed per hour, under the assumption that a library needs to be

[2] Metrohm Raman's firmware and software no longer supports this version of the CBEx. The current version of the CBEx, which is support and also ruggedized costs $18,500

developed prior to analysis for each new sample being analyzed[3]. Over the course of 10,000 working hours this equates to 200,000 total samples from 1,000 different medicines. Dividing this by the list price of the unit ($18,500) equals $0.0925 per sample.

2.2. Performance Evaluation

Acronyms and Definitions	
AAC	Acetaminophen + Acetylsalicylic + Caffeine liquid capsules
AC1	Acetaminophen liquid capsules, brand 1
AC2	Acetaminophen liquid capsules, brand 2
ACR	USP Acetaminophen Reference Standard
AL1	Artemether + Lumefantrine tablets, brand 1
AL2	Artemether + Lumefantrine tablets, brand 2, lot 1
AL3	Artemether + Lumefantrine tablets, brand 2, lot 2
AL4	Artemether + Lumefantrine tablets, brand 1, lot 2
AL5	Artemether + Lumefantrine tablets, brand 1, lot 3
AMR	USP Amoxicillin Reference Standard
AMX1	Amoxicillin capsules, brand 1
AMX2	Amoxicillin capsules, brand 2
ARR	USP Artemether Reference Standard
CBEx	CBEx
DNC	Did Not Collect
EC	Environmental Chamber exposure
ETR	USP Ethambutol Reference Standard
FSR	USP Furosemide Reference Standard
FSM1	Furosemide injection, brand 1
FSM2	Furosemide injection, brand 2
INR	USP Isoniazid Reference Standard
LUR	USP Lumefantrine Reference Standard
OXR	USP Oxytocin Reference Standard
OXY1	Oxytocin injection, brand 1
OXY2	Oxytocin injection, brand 2
PS	Point and shoot adapter
PYR	USP Pyrazinamide Reference Standard
RH1	Rifampicin + Isoniazid tablets, brand 1
RH2	Rifampicin + Isoniazid tablets, brand 2
RHZE1	Rifampicin + Isoniazid + Pyrazinamide + Ethambutol tablets, brand 1
RHZE2	Rifampicin + Isoniazid + Pyrazinamide + Ethambutol tablets, brand 2
RIR	USP Rifampin Reference Standard
RS	USP Reference Standard
RV	Raman vial

Additional details of samples, standards, and equipment used can be found in Annex 1 and Annex 2.

[3] Ordinarily, one large, multi-sample library can be developed at once and used indefinitely provided the spectra are frequently verified against the reference sample. However, for the purposes of this cost per test calculation, a more conservative approach was used to account for different levels of expertise across users

CBEx Operating Procedure

1. Switch on instrument and perform daily verification using the calibration function and the manufacturer provided calibration standard.
2. The following software parameters were selected for analysis:
    a. Data was collected at "power level 5", specified by the manufacturer to be 50 mW (± 3 mW) with a spot size of 0.2 – 2.5 mm, unless otherwise specified.
    b. Data was collected over the Raman shift range of 400 to 2300 cm^{-1} with a manufacturer specified resolution of 12-14 cm^{-1}.
    c. All spectra were collected using "reference" and "baseline" features.
    d. All spectra were collected using the "auto-integration" feature, which optimizes data acquisition time (t_{acq}).
3. Identify appropriate adapter (e.g. vial holder, point and shoot adapter, SERS adapter) for analysis.
4. Collect reference spectra[4] in the library management window using the appropriate adapter and via a PC connection (rather than in standalone mode) using PEAK software version and save as .rmn files.
    a. Collect tablet drug product spectra using the "point and shoot" (PS) adapter, which was adjusted to position the samples at an instrument focal length of 8 mm.
    b. Deposit powdered drug products, solutions or reference standards spectra in manufacturer provided vials and analyze using the inbuilt vial holder.
    c. Collect injection drug product spectra using the vial holder or SERS adapter and manufacturer provided SERS substrates[5].
5. Collect sample spectra in the acquisition window and compare against spectra in the reference library window via the match score.

Sample Preparation

Reference Standards

All Reference Standards (RS) were obtained from USP. RS were analyzed as is and, in addition, FSR and OXR were analyzed as prepared solutions. The FSR solution was prepared by dissolving FSR in a sodium hydroxide solution to obtain a final concentration no greater than 10 mg/mL. The OXR solution was prepared by dissolving OXR in water to obtain a final concentration of 10 units/mL.

Drug products

Drug products were analyzed both as is and following minimal sample preparation (see Figure 1). Tablets were prepared as a powder after grinding with a pestle and mortar. Powder-filled capsules were prepared by emptying the content of the capsule, grinding the contents using a

[4] Multiple scans of each product were collected and were observed to be identical, provided the software parameters were kept constant. Therefore, only one scan was used for each reference and sample spectra during analysis

[5] SERS substrates can be purchased through Diagnostic Ansers. The substrates (gold or silver) are ink-jet printed on paper strips sealed on clear slides.

pestle and mortar, and emptying the contents into a vial. Liquid gel capsules were prepared by empting the content of the capsules into a vial. Injections were prepared by either emptying the contents into a vial or submerging a silver (Ag) or gold (Au) SERS substrate in the samples for 3 hours.

Figure 1. Sample types investigated and drug product preparation schemes

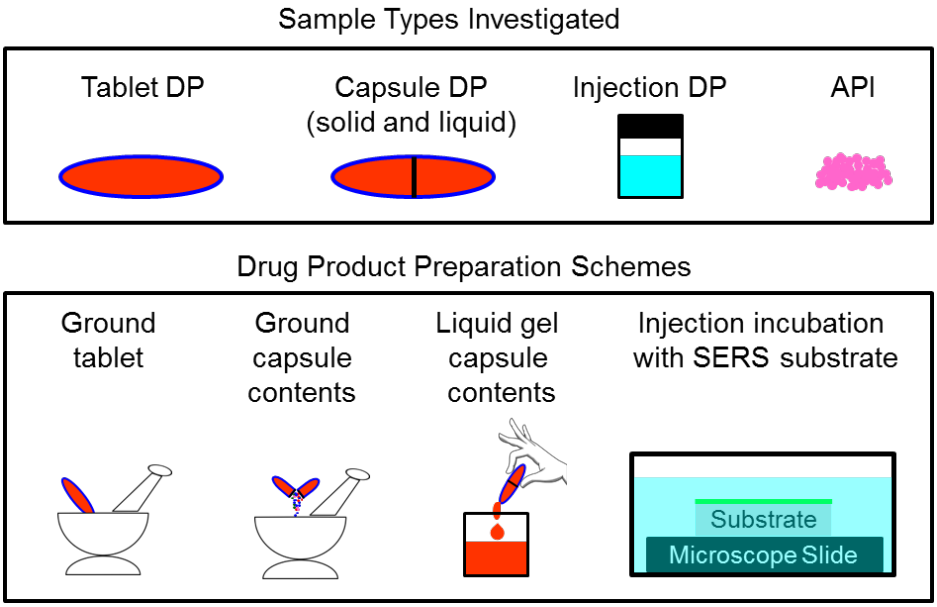
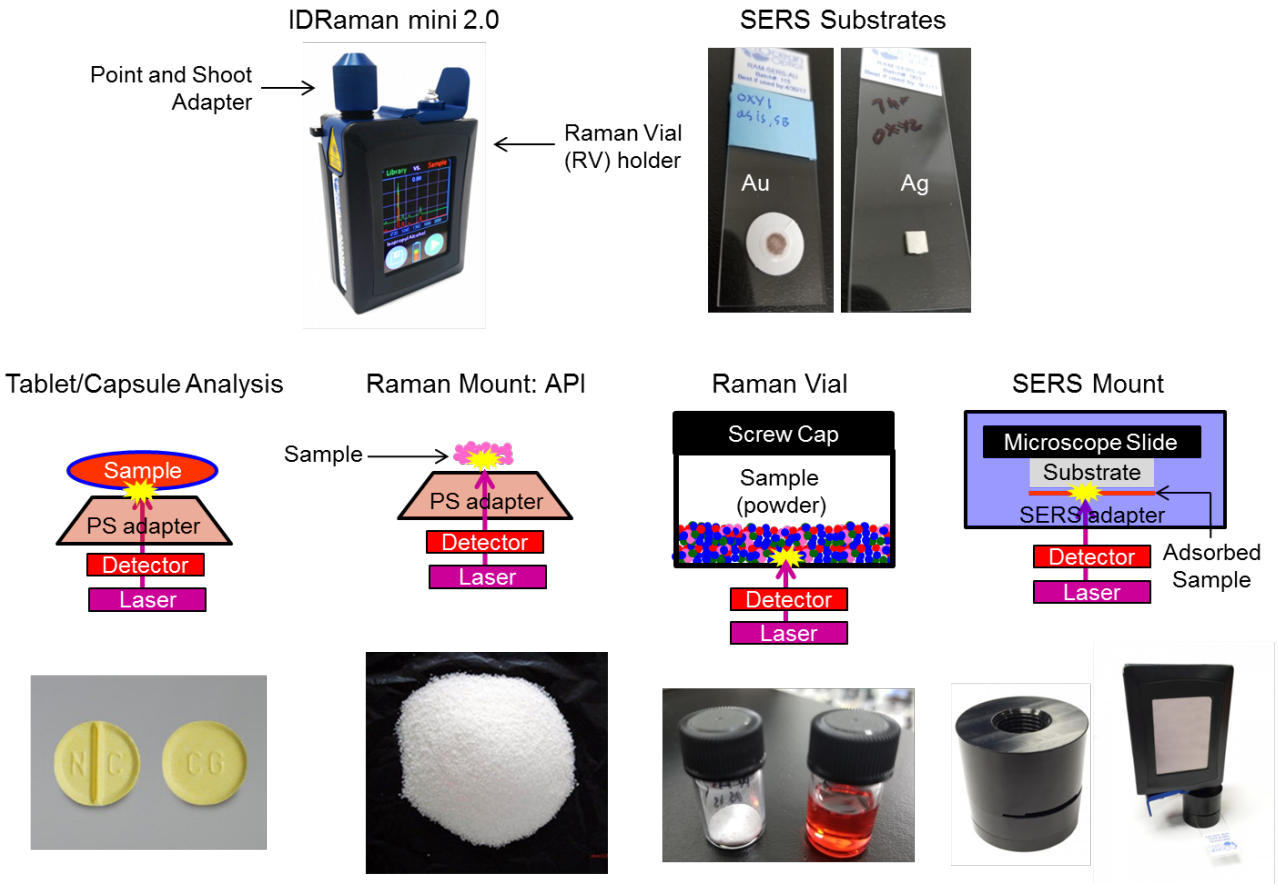


Figure 2. Raman spectroscopy equipment and analysis conditions



Degraded Samples

An environmental chamber or oven was used to 'degrade' some samples before analysis to mimic substandard medicines. The conditions for both are below:

- Environmental Chamber condition 1: 85^{0}C / 85% relative humidity for 7 hours
- Environmental Chamber condition 2: 85^{0}C / 85% relative humidity for 24 hours
- Environmental Chamber condition 3: 85^{0}C / 85% relative humidity for 96 hours
- Oven condition 1: 105^{0}C for 3 hours
- Oven condition 2: 105^{0}C for 6 hours
- Oven condition 3: 105^{0}C for 15 hours

Match Score

Raman spectra were compared using "Match Scores" generated by the PEAK software. A Match Score is a similarity or hit quality index (HQI) calculated using Euclidean distance that ranges between 0 and 1, where 1 describes identical spectra and 0 describes spectra with no similarity. It is important to note that although match scores are not based on probability or confidence levels, only scores above 0.85 are reported by the instrument when used in standalone mode. This is a vendor specified limit, which is considered an identification threshold.

High-Performance Liquid Chromatography (HPLC) Conditions

An Agilent 1290 Infinity series HPLC operated by Waters Empower 3 Software outfitted with an Agilent Zorbax SB-CN column (5 µm, 4.6 × 250 mm) was used for HPLC data collection. The procedure used for chromatographic analysis was the final version of the artemether + lumefantrine tablets monograph from the USP Medicines Compendium. The flow rate, column temperature, and injection volume were 0.8 mL/min, 30°C, and 20 µL, respectively. Chromatographic peaks for AR and LU were detected at 210 nm and 380 nm, respectively.

Methodology Limitations

Certain limitations were encountered during this performance review, which were inevitable given the nature of the technology and the objectives of the review. They are identified below:

1. Eight different drug products samples were analyzed along with their respective reference standards. Although all products are on the WHO's Essential Medicines List [3], they represent only a small fraction of the list. Ideally, many more samples would be analyzed, particularly as not all APIs are Raman active. However, these eight samples deliberately represented a variety of therapeutic indications, dosage forms, and dosage strengths to enable broader conclusions about the utility of the CBEx to be made.
2. No actual substandard or falsified medicines were obtained for the evaluation. Instead genuine products were degraded in an environmental chamber or oven, or different dosage strengths were used to mimic substandard or falsified medicines. Although not ideal, the data obtained using these methods were able to provide information on the ability of the instrument to identify poor quality medicines. Future evaluations could look at the possibility of collaborating with genuine manufacturers to obtain placebo (no API) or low dose versions of their products or use reference standards and public ingredient information to formulate substandard or falsified medicines.
3. Chemical analyses of excipient profiles of different brands of the same product were not performed. Future work could perform such an evaluation to determine whether minor match score differences between brands are due to variances in excipient profiles.

3. Results

3.1. General Information

Data

Currently, Metrohm Raman can provide the hardware and software only in English. There is a four-digit code lock on the instrument when first turning the instrument on and an internal timer can be set to lock out a user after a set time period. There are no permission requirements using the software on a computer. The instrument does not have internet or Bluetooth capabilities. Although data cannot be directly transferred between instruments, it can be easily transferred from an instrument onto a computer using the micro-USB cable and then onto a second instrument. When collecting and transferring spectra, three file types are automatically created:

- .rmn file (used to view spectra in the PEAK software)
- PKCS #7 certificates
- Text document (for further external processing using other software)

Access, Handling, Maintenance, and Repair

CBEx is commercially available globally but can only be purchased directly from Metrohm Raman. While issues can be diagnosed over the phone, all service and repairs are provided by Metrohm Raman offices in Lararmie, Wyoming, USA. Calibration standards have a two year lifetime and are labeled with an expiration date. The instrument should be calibrated daily, prior to use.

Durability

CBEx is not waterproof or completely sealed so dust ingress is possible. The operating range of the instrument is -10ºC to 40ºC and can operate in up to 95% non-condensing humidity. Ambient light can cause instrument response issues; however the referencing function generally alleviates these issues. Although the standard CBEx is not ruggedized, a ruggedized version is commercially available.

Use

CBEx can theoretically analyze any liquids and solids provided they have Raman active molecules. Spectra are collected in a maximum of five seconds and only one sample can be analyzed at a time.

Further details as well as the instrument brochure can be found at the following website: http://www.wysri.com/cbex/

3.2. Performance Evaluation

Application II: Identification of Bulk Drug Substances or Active Pharmaceutical Ingredients in Finished Pharmaceutical Products

All data below were collected between April 2017 and August 2017. Application II is per the USP Stimuli to the Revision Process: Evaluation of Screening Technologies for Assessing Medicine Quality [4].

CBEx is a handheld Raman spectrometer, which uses a 785 nm laser to generate spectral signatures of medicines based on their levels of Raman scattering. The x-axis of the spectra is presented as wavenumbers (cm^{-1}) while the y-axis is presented as arbitrary intensity. Spectra can be compared against one another via the match score to determine the level of agreement between, for example, a genuine and a suspected falsified medicine. Spectral libraries can also be developed to screen an unknown = medicine against multiple reference spectra.

Apart from the initial reproducibility, reliability, background and raster studies, which used four samples (an artemether + lumefantrine tablet, lumefantrine reference standard, an amoxicillin capsule and amoxicillin reference standard) results will be presented by sample rather than by analytical parameter (e.g. specificity). This is because like most spectroscopic techniques, Raman spectra are specific to a medicine. The samples selected to evaluate the capabilities of the instrument are all products from the current WHO Essential Medicines List and represent different therapeutic indications, dosage forms and dosage strengths.

Reproducibility and Reliability

Table 2 provides a match score comparison between instruments. Individual spectra were collected for all four samples on all three instruments using either the Raman vial or the point and shoot adapter. Data was transferred via the PEAK software to a PC to enable comparison of spectra from different instruments. Match scores were then determined by comparing the spectra of an instrument against the spectrum of the 'reference' instrument. For example, when CBEx2 was used as the comparator instrument, the match scores for the spectra collected on CBEx3 and CBEx4 using AL1 were 0.94 and 0.92, respectively, indicating strong agreement.

Table 2. Match Score Comparisons Between Three Instruments Using Four Different Samples and Two Analysis Types

Reference Instrument	Sample	CBEx2	CBEx3	CBEx4	Adapter
CBEx2	AL1	N/A[6]	0.94	0.92	PS
	LUR	N/A	0.95	DNC[7]	RV
	AMX1	N/A	0.94	0.97	PS
	AMR	N/A	0.97	0.97	RV
CBEx3	AL1	0.94	N/A	0.91	PS
	LUR	0.95	N/A	DNC	RV
	AMX1	0.94	N/A	0.98	PS
	AMR	0.97	N/A	0.97	RV
CBEx4	AL1	0.92	0.91	N/A	PS
	LUR	DNC	DNC	N/A	RV
	AMX1	0.97	0.98	N/A	PS
	AMR	0.97	0.97	N/A	RV

The lowest match score for this work was 0.91 when using CBEx3 as the comparator instrument and analyzing AL1 on CBEx4 and vice versa. Figure 5 (Annex 3) shows an overlay of the three AMX1 spectra.

Table 3 shows the day to day variability of spectra for three consecutive days on IDR2 for two different samples using both the point and shoot adapter and the Raman vial.

Table 3. Day to Day Variability on CBEx2

Reference Day	Sample	Day 1	Day 2	Day 3	Adapter
Day 1	AL4	N/A	1.00	1.00	PS
	AMX1	N/A	1.00	1.00	RV
Day 2	AL4	1.00	N/A	1.00	PS
	AMX1	1.00	N/A	1.00	RV
Day 3	AL4	1.00	1.00	N/A	PS
	AMX1	1.00	1.00	N/A	RV

[6] Results are not applicable because the comparator spectrum is being compared against itself, generating a match score of 1.00

[7] DNC – did not collect

Match scores for all conditions was 1.00. Expanding upon table 3, table 4 provides spectra variation between calibrations on the same day to demonstrate the intermediate precision of the instrument. The same sample was analyzed three times. The instrument was calibrated each time before the sample was analyzed. The data sets in tables 3 and 4 were collected to demonstrate that spectra are independent of time and calibration.

Table 4. Calibration to calibration variability on CBEx2

Reference Calibration	Sample	Cal 1	Cal 2	Cal 3	Adapter
Cal 1	AMX1	N/A	0.99	0.99	RV
Cal 2	AMX1	0.99	N/A	0.99	RV
Cal 3	AMX1	0.99	0.99	N/A	RV

Background and Raster

Table 5 compares the spectra of AL1 and AMX1 with the orbital raster scanning and background correction functionalities on and off. Raster scanning is a technique that maintains laser resolution while scanning a larger area of the sample, theoretically producing a more representative spectrum.

Table 5. Background and Raster Comparison

Reference Sample	AL4 (B)	AL4 (NB)	AL4 (R)	AL4 (NR)	AMX1 (B)	AMX1 (NB)	AMX1 (R)	AMX1 (NR)	Adapter
AL4 (B)	N/A	0.14	1.00	1.00	N/A	N/A	N/A	N/A	PS
AL4 (NB)	0.14	N/A	0.14	0.14	N/A	N/A	N/A	N/A	PS
AL4 (R)	1.00	0.14	N/A	1.00	N/A	N/A	N/A	N/A	PS
AL4 (NR)	1.00	0.14	1.00	N/A	N/A	N/A	N/A	N/A	PS
AMX1 (B)	N/A	N/A	N/A	N/A	N/A	0.16	1.00	0.90	RV
AMX1 (NB)	N/A	N/A	N/A	N/A	0.16	N/A	0.16	0.24	RV
AMX1 (R)	N/A	N/A	N/A	N/A	1.00	0.16	N/A	0.90	RV
AMX1 (NR)	N/A	N/A	N/A	N/A	0.90	0.24	0.90	N/A	RV

Baselined, raster, and non-raster spectra of AL4 matched well or showed good agreement between spectra (1.00). Baseline, raster, and non-raster spectra of AMX1 also show good agreement, although the comparison of raster and non-raster spectra have a matching score of 0.90 instead of 1.00 as seen for AL4. Baselined and non-baselined spectra of both AL4 and AMX1 show no agreement. Figure 6 (Annex 1) shows a spectrum comparison between baselined and non-baselined AL4 and AMX1.

Artemether + Lumefantrine tablets

Table 6 compares the spectra of two different brands, two different lots, two different dosage strengths, three different 'preparations' of artemether + lumefantrine tablets and the two constituent reference standards. AL1 and AL4 are two different batches of 20 mg / 120 mg tablets from one manufacturer. AL2 and AL3 are two different batches of 20 mg / 120 mg tablets from a second manufacturer.

Table 6. Brand, Preparation, and Reference Standard Comparison

Reference Sample	AL1	AL2	AL3	AL4[1]	AL4[2]	AL4[3]	ARR	LUR	Adapter
AL1 (tablet, brand 1)	N/A	0.99	1.00	0.99	0.99	0.99	0.00	0.99	PS
AL2 (tablet, brand 2, lot 1)	0.99	N/A	0.99	1.00	1.00	0.99	0.00	0.98	PS
AL3 (tablet, brand 2, lot 2)	1.00	0.99	N/A	0.99	0.99	0.99	0.00	0.99	PS
AL4[1] (through primary packaging)	0.99	1.00	0.99	N/A	1.00	0.99	0.00	0.97	PS
AL4[2] (tablet, brand 1, lot 2)	0.99	1.00	0.99	1.00	N/A	0.99	0.00	0.98	PS
AL4[3] (powder)	0.99	0.99	0.99	0.99	0.99	N/A	0.00	0.98	RV
ARR	0.00	0.00	0.00	0.00	0.00	0.00	N/A	0.00	RV
LUR	0.99	0.98	0.99	0.97	0.98	0.98	0.00	N/A	RV

Except for the artemether reference standard, the match score for all samples and conditions was between 0.97 and 1.00. Based on these match scores, there was good agreement between different brands, different batches of the same brand, and powdered or as is tablets. The primary packaging of one of AL4 also did not affect the spectra. However, the spectral signature of the artemether + lumefantrine tablets is dominated by lumefantrine and has no contribution from artemether. The lack of artemether contribution is likely why the match score between ARR and all the AL samples was 0.00. Figure 7 (Annex 3) shows an overlay of spectra for AL3, AL4, ARR, and LUR using AL3 as the reference spectra.

Table 7 compares match scores for an AL3 sample that has been degraded under different conditions along with a second artemether + lumefantrine tablet sample with a higher dosage strength than the degraded sample: 80 mg / 480 mg for AL5 compared to 20 mg / 120 mg for AL3. HPLC assay values for four of the degraded conditions are also included.

Table 7. Dosage Strength and Degradation Comparison

Reference Sample	AL3 (powder)	AL3 (105 3 h)	AL3 (105 6h)	AL3 (105 15h)	AL3 (85/85 7h)	AL3 (85/85 24h)	AL5	Adapter
AL3 (powder)	N/A	0.96	0.92	0.90	0.97	0.82	0.99	RV
AL3 (105[8] 3h)	0.96	N/A	0.98	0.97	0.96	0.91	0.96	RV
AL3 (105 6h)	0.92	0.98	N/A	0.99	0.94	0.96	0.91	RV
AL3 (105 15h)	0.90	0.97	0.99	N/A	0.93	0.97	0.90	RV
AL3 (85/85[9] 7h)	0.97	0.96	0.94	0.93	N/A	0.89	0.96	RV
AL3 (85/85 24h)	0.82	0.91	0.96	0.97	0.89	N/A	0.81	RV
AL5 (tablet)	0.99	0.96	0.91	0.90	0.96	0.81	N/A	PS
HPLC Assay (AR)	102%	78%	61%	33%	DNC	DNC	DNC	N/A
HPLC Assay (LU)	108%	102%	104%	100%	DNC	DNC	DNC	N/A

There is good agreement (match score of 0.99) between the two non-degraded samples of different dosage strengths, one of which had four times more APIs than the other. Match scores of degraded samples compared against the unexposed powder, decreased with increasing exposure. This coincided with a decrease in the assay values of both APIs for all three oven

[8] Temperature (celsius)
[9] Percent relative humidity

conditions. For example, the unexposed sample, which was used as the reference spectra, had assay values of 102% and 108% for artemether and lumefantrine, respectively. When compared against the same sample exposed to oven condition 3 (105^0C for 15 hours), the match score was 0.90 while the assay values dropped to 33% and 100% for artemether (AR) and lumefantrine (LU), respectively with the decrease in lumefantrine being insignificant. The instrument identified the exposed sample as matching that of the reference powder even though the content of artemether had decreased 67%. Figure 8 shows an overlay of $AL3^1$, $AL3^2$, $AL3^4$ and AL5 using $AL3^1$ as the reference spectra.

Rifampicin + Isoniazid + Pyrazinamide + Ethambutol tablets

Table 8 compares the spectra of two different brands, and three different preparations of rifampicin + isoniazid + pyrazinamide + ethambutol tablets and the four constituent reference standards. RHZE1 and RHZE2 are both 150 mg + 75 mg + 400 mg + 275 mg tablets from two different manufacturers.

Table 8. Brand, Preparation, and Reference Standard Comparison

Reference Sample	ETR	INR	PYR	RHZE1 (tablet)	RHZE1 (powder)	RHZE2 (packaging)	RHZE2 (tablet)	RHZE2 (powder)	RIR	Adapter
ETR	N/A	0.00	0.18	0.00	0.10	0.00	0.00	0.11	0.03	RV
INR	0.00	N/A	0.00	0.00	0.29	0.00	0.00	0.24	0.32	RV
PYR	0.18	0.00	N/A	0.00	0.12	0.00	0.00	0.21	0.02	RV
RHZE1 (tablet)	0.00	0.00	0.00	N/A	0.01	0.91	0.89	0.00	0.01	PS
RHZE1 (powder)	0.10	0.29	0.12	0.01	N/A	0.00	0.00	0.97	0.93	RV
RHZE2 (through primary packing)	0.00	0.00	0.00	0.91	0.00	N/A	0.93	0.00	0.00	PS
RHZE2 (tablet)	0.00	0.00	0.00	0.89	0.00	0.93	N/A	0.00	0.00	PS
RHZE2 (powder)	0.11	0.24	0.21	0.00	0.97	0.00	0.00	N/A	0.86	RV
RIR	0.03	0.32	0.02	0.01	0.93	0.00	0.00	0.86	N/A	RV

Two brands show good agreement when powdered (0.97). The slight 0.03 variation is likely the result of manufacturer to manufacturer variability. There is moderate agreement between the two brands when analyzed as tablets (0.89) or through packaging (0.91). However, there is no agreement (RHZE1=0.01, RHZE2=0.00) between the tablet and powder of the same sample, which is most the result of the tablet coating. The rifampin reference standard and the powders of the two brands show moderate agreement (0.93 and 0.86). However, there is also almost no agreement between the other three constituent reference standards and the powders of the two brands. For example, using RHZE1 powder as the reference sample, the match scores are 0.10, 0.29 and 0.12 for ethambutol, isoniazid and pyrazinamide, respectively when comparing to the remaining three reference standards. Figures 9 and 10 (Annex 3) show a comparison of RHZE1 powder and tablet and RHZE1 powder compared against the four reference standards, respectively.

Table 9 compares the spectra of two brands of rifampin + isoniazid tablets, one brand of rifampin + isoniazid + pyrazinamide + ethambutol tablets, and rifampin, and isoniazid reference standards.

Table 9. Degradation and Fixed Dose Combination Comparison

Reference Sample	INR	RH1 (tablet)	RH1 (powder)	RH2 (powder)	RHZE1 (powder)	RHZE1 (85/85 7h)	RIR	Adapter
INR	N/A	0.02	0.39	0.46	0.29	0.20	0.32	RV
RH1 (tablet)	0.02	N/A	0.03	0.03	0.05	0.02	0.05	PS
RH1 (powder)	0.39	0.03	N/A	0.94	0.88	0.50	0.92	RV
RH2 (powder)	0.46	0.03	0.94	N/A	0.91	0.58	0.96	RV
RHZE1 (powder)	0.29	0.05	0.88	0.91	N/A	0.67	0.93	RV
RHZE1 (85/85 7h)	0.20	0.02	0.50	0.58	0.67	N/A	0.54	RV
RIR	0.32	0.05	0.92	0.96	0.93	0.54	N/A	RV

There is good agreement between the RH1 and RH2 (0.94), RH1 and RIR (0.92) and RH2 and RIR (0.96). There is also moderate agreement between RHZE1 (0.88) and RH1 (0.88) and RH2 (0.91). There is no agreement between the degraded RHZE1 sample and the non-degraded RHZE1 sample (0.67). Figure 11 (Annex 3) shows a comparison between the RH2, RHZE1 powder and the degraded RHZE1.

Amoxicillin Capsules

Table 10 compares the spectra of two brands of amoxicillin capsules (AMX1 and AMX2), degraded AMX1, and an amoxicillin reference standard (AMR).

Table 10. Brand, Degradation, Preparation, and Reference Standard Comparison

Reference Sample	AMR	AMX1 (capsule)	AMX1 (powder)	AMX1 (85/85 7h)	AMX2 (powder)	Adapter
AMR	N/A	0.10	0.99	0.67	0.99	RV
AMX1 (capsule)	0.10	N/A	0.10	0.17	0.09	PS
AMX1 (powder)	0.99	0.10	N/A	0.71	1.00	RV
AMX1 (85/85 7h)	0.67	0.17	0.71	N/A	0.70	RV
AMX2 (powder)	0.99	0.09	1.00	0.70	N/A	RV

There is good agreement between AMX1 and AMX2 (1.00) and AMR (0.99) when the powder of the sample rather than the coloured capsule shell is analyzed. Using the Raman vial instead of the point and shoot adapter may also contribute to higher match scores. The AMX1 powder and the degraded powder showed marginal agreement (0.71).

Acetaminophen Liquid Capsules

Table 11 compares the spectra of two brands of acetaminophen liquid capsules (AC1 and AC2), degraded AC2, one brand of acetaminophen + aspirin + caffeine liquid capsules (AAC), and acetaminophen reference standard (ACR).

Table 11. Brand, Degradation, Preparation and Reference Standard Comparison

Reference Sample	AC1 (capsule)	AC1 (liquid)	AC2 (85/85 96h)	AC2 (liquid)	AAC (capsule)	AAC (liquid)	ACR	Adapter
AC1 (capsule)	N/A	0.89	0.60	0.90	0.00	0.31	0.54	PS
AC1 (liquid)	0.89	N/A	0.47	0.98	0.00	0.34	0.56	RV

AC2 (85/85 96h)	0.60	0.47	N/A	0.50	0.21	0.14	0.27	RV
AC2 (liquid)	0.90	0.98	0.50	N/A	0.00	0.35	0.59	RV
AAC (capsule)	0.00	0.00	0.21	0.00	N/A	0.02	0.00	PS
AAC (liquid)	0.31	0.34	0.14	0.35	0.02	N/A	0.60	RV
ACR	0.54	0.56	0.27	0.59	0.00	0.60	N/A	RV

When comparing the liquids, there is good agreement between AC1 and AC2 (0.98), moderate agreement when comparing the AC1 capsules to the liquid (0.89), and very little agreement between either AC1, AC2, or AAC and ACR (0.56, 0.59 and 0.60, respectively). There was no agreement (0.50) between the unexposed AC2 liquid and the same degraded liquid. Figure 12 (Annex 3) compares the spectra of the AC1 capsule, AC1 liquid, degraded AC2, AAC liquid, and ACR

Oxytocin and Furosemide Injections

Table 12 compares two brands of furosemide injection (FSM1 and FSM2), degraded FSM1 and furosemide reference standard (FSR).

Table 12. Furosemide Brand, Degraded and Reference Standard Comparison

Reference Sample	FSM1 (liquid)	FSM2 (liquid)	FSM2 (85/85 96h)	FSR (powder)	Adapter
FSM1 (liquid)	N/A	0.98	0.98	0.20	RV
FSM2 (liquid)	0.98	N/A	0.98	0.22	RV
FSM2 (85/85 96h)	0.98	0.98	N/A	0.21	RV
FSR (powder)	0.20	0.22	0.21	N/A	RV

There is good agreement between FSM1 and FSM2 (0.98). However, there was also good agreement between FSM2 and the degraded FSM2 (0.98) but no agreement between FSR and the injection samples.

Table 13 compares two brands of oxytocin injection (OXY1 and OXY2), degraded OXY1, OXY2 incubated SERS substrate, and oxytocin reference standard (OXR). Because oxytocin is found in very small concentrations in injections, the samples were also analyzed using SERS substrates to determine whether the substrates could enhance the Oxytocin Raman signal.

Table 13. Oxytocin Brand, Degraded, Reference Standard, and SERS Comparison

Reference Sample	BAg	BAu	OXR (liquid)	OXY1 (liquid)	OXY1 (85/85 96h)	OXY1 (SAG)	OXY1 (SAU)	OXY2 (liquid)	Adapter
Blank (SAG)	N/A	0.59	0.01	0.01	0.01	0.01	0.60	0.00	SERS
Blank (SAU)	0.59	N/A	0.00	0.00	0.00	0.00	0.95	0.00	SERS
OXR (liquid)	0.01	0.00	N/A	0.64	0.65	0.40	0.00	0.52	RV
OXY1 (liquid)	0.01	0.00	0.64	N/A	0.96	0.30	0.00	0.94	RV
OXY1 (85/85 96h)	0.01	0.00	0.65	0.96	N/A	0.28	0.00	0.93	RV
OXY1 (SAG)	0.01	0.00	0.40	0.30	0.28	N/A	0.00	0.26	SERS
OXY1 (SAU)	0.60	0.95	0.00	0.00	0.00	0.00	N/A	0.00	SERS
OXY2 (liquid)	0.00	0.00	0.52	0.94	0.93	0.26	0.00	N/A	RV

There is good agreement between OXY1 and OXY2 (0.94) and the degraded OXY1 (0.96) but no agreement between OXR and the injection samples. Using either the Ag (0.40) or Au (0.00) SERS substrate had no impact on the agreement with OXR and OXY1.. There was good agreement between the blank Au SERS substrate and OXY1 incubated Au SERS substrate (0.95), indicating that Oxytocin has little impact on the overall spectra. Figure 14 (Annex 3) shows a spectral comparison between OXR, OXY1 (liquid) and OXY (SAU).

3.3. Field Evaluation

This field evaluation performed in India and Zimbabwe in August 2017 reviewed two major parameters: training requirements and field utility. India and Zimbabwe were selected because they represent two countries with different regulatory environments, where screening technologies have not been used extensively in the past but have the potential to be deployed effectively to combat substandard and falsified medicines.

Training Requirements

This first component of the field evaluation involved working with and training local staff in India and Zimbabwe to assess the amount of training required to enable staff to reliably and productively utilize CBEx in the field. The training involved 5 full days of work, which included 2 days of hands-on and theoretical work followed by 2 days in the field collecting and testing samples. Across both countries, 10 total staff from the Telangana Drug Control Authority and Medicines Control Authority of Zimbabwe were trained; of these, 6 were laboratory staff (either microbiologists or chemists) and 4 were inspectors. To evaluate the perceived training timeframes for three levels of use of the instrument (basic, intermediate, and advanced), two data sources were used to develop a training timeframe requirements matrix: (1) a survey completed by trainees following the training and (2) the trainer observations. Two variables were used to develop the matrix:

1. User experience (prior to training):
    a. *Non-technical experience*: A trainee with no prior laboratory experience and no background in one of the physical sciences (e.g., chemistry, biology).
    b. *Technical experience*: A trainee with prior experience working in a laboratory and/or a background in one of the physical sciences.
    c. *Specialized experience*: A trainee with theoretical and practical experience utilizing the technology or the technique underpinning the technology.
2. User type[10] (following training):
    a. *Basic user*: A user with the ability to follow a standard operating procedure or work instruction to set up and run the instrument and collect data.
    b. *Intermediate user*: A user with the ability to develop and modify methods and evaluate and interpret results.

[10] The user type abilities build upon the previous level (e.g., an advanced user can perform the functions of an advanced user as well as a basic and intermediate user).

c. *Advanced user*: A user with the ability to train other staff and perform basic troubleshooting.

Table 2 provides recommended training timeframes for trainees to reach one of three user levels—basic, intermediate, or advanced—based on the performance evaluation, field evaluation, survey given to trainees and local staff, and trainer observations.

Table 12: Training Timeframe Requirements

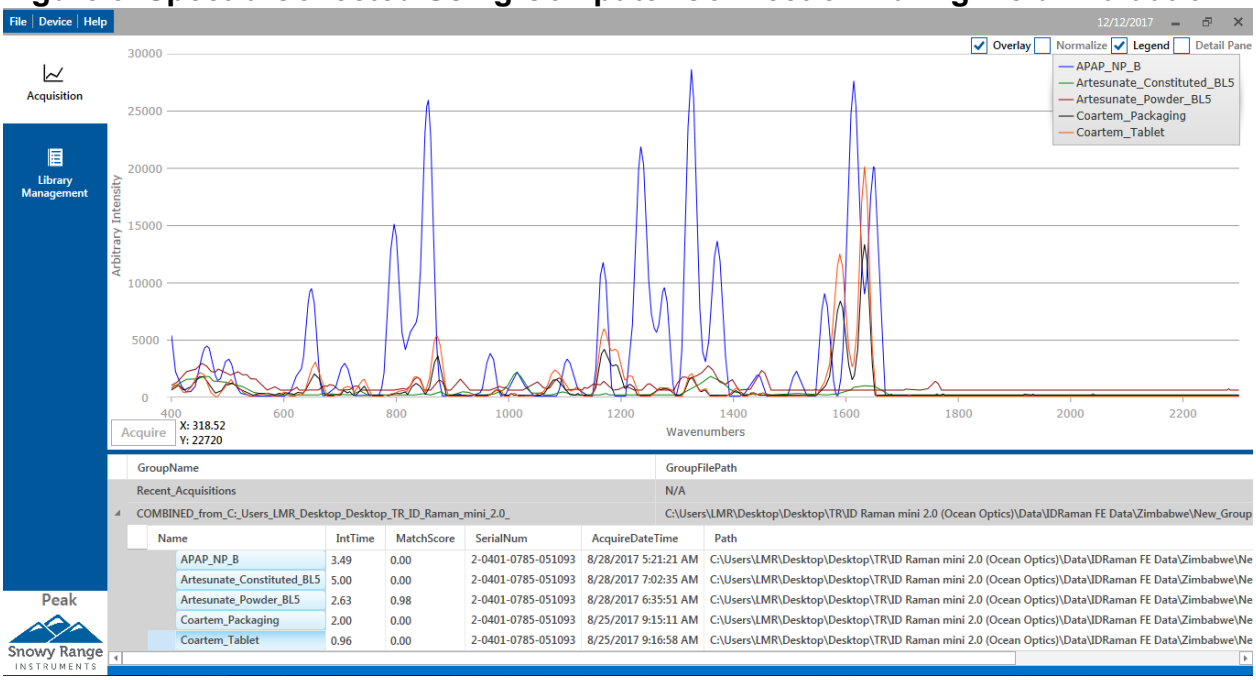
User Experience	User Type		
	Basic	***Intermediate***	***Advanced***
Non-technical	Between 1 and 2 days	1 week	2 weeks
Technical	1 day	Between 1 day and 1 week	1 to 2 weeks
Specialized	1 to 2 hours	1 day	Less than 1 week

Field Utility

The second component of the field evaluation involved running samples using CBEx in field settings and determining the utility of the instrument in these environments. It also included identifying any challenges associated with traveling with CBEx.

No problems were encountered during routine international air transportation, which included security checks and hand and checked luggage storage on long-haul flights. Travel by vehicle to various sampling sites also did not involve any challenges, and the instrument withstood temperatures between room temperature and approximately 40 degrees Celsius. The rugged travel case and small overall form factor of the instrument made transport convenient. The instrument was also dropped accidentally on two occasions during the field evaluation. These drops did not alter the observed functioning of the instrument. The instrument ran for approximately three hours and collected a scan every five minutes before the batteries needed to be replaced. Scans were collected in a maximum of five seconds and the transfer of spectra and libraries between instruments using a PC was quick and simple. Spectral libraries were developed at the training venues in both countries and the instrument was taken to informal markets, rural health outlets, pharmacies, and wholesalers where samples were collected and analyzed onsite. Trainees completed this work themselves and no issues were encountered in the collection or interpretation of data. Figure 3 provides an example of several spectra collected during the training while figure 4 shows a comparison of two amoxicillin spectra when using the instrument in standalone mode.

Figure 3. Spectra Collected Using Computer Connection During Field Evaluation



One of the instruments encountered an issue with the screen during initial field evaluation work in India. Output on the screen became a faint red and the text and figures on the interface were difficult to make out. The instrument was still able to function when connected to a PC but using it in standalone mode was challenging. The vendor was contacted by email, responded within 24 hours and diagnosed the problem (an internal disconnected wire) remotely.

Figure 4. Result of an Amoxicillin Sample Analysis in the Field in Zimbabwe

4. Review and Conclusions

4.1. Performance Evaluation

There was no day to day and calibration to calibration variability between spectra and only marginal instrument to instrument variability, indicating that the CBEx provides reproducible data (see tables 2, 3 and 4) independent of time. Furthermore, the baseline function of the instrument is an effective feature that helps mitigate the effects of sample fluorescence on a spectrum (see Annex 3, figure 6), an overarching limitation of Raman spectroscopy.

Results overwhelmingly indicated that the instrument cannot distinguish between brands of the same medicine with good agreement being seen when comparing different brands for all eight samples. This seems to imply that the difference in excipient profiles between brands do not have strong enough Raman cross sections to differentiate brands. While this is not an issue when only the quality of a medicine is being evaluated, it may present a challenge for manufacturers or procurement agencies looking to authenticate suspect versions of their produced or procured products. The deterioration of the Raman spectrum that is observed for AL, AMX, AC, AAC, RHZE and RH with increased exposure in the EC seems to indicate increased fluorescence interference, likely due to degradation products. This implies that a degraded sample of several of these products could be identified as substandard, depending somewhat on the level of degradation. Further work would however need to be done to ascertain the threshold (amount of degradation) at which a substandard product would still be identified by the CBEx and would also need to be corroborated using confirmatory analysis to assay the content of API and degradation products.

When analyzing AL samples, there was good agreement between the spectra of the AL powder measured in the RV, the AL tablet measured using the PS adapter, and the AL tablet analyzed through the primary packaging using the PS adapter. This indicates that an AL method could be developed that would not require destruction of the sample. However, the CBEx was not able to distinguish between different dosage strengths of AL tablets, specifically 20 mg / 120 mg AL tablets and 80 / 480 mg AL tablets. Agreement between these two samples was 0.99 per table 6. This indirectly implies that a substandard medicine with less than the required amount of API would be incorrectly identified as an AL sample containing both APIs. Secondly, the AL tablet spectra is dominated by lumefantrine with seemingly no contribution from artemether, as evidenced by the match scores between AL and ARR (0.00) and AL and LUR (0.98). This observation was reinforced when analyzing exposed samples. The match score and artemether API assay value of the product did decrease when exposed and while there was a clear trend for artemether with respect to increasing exposure time, there was no such trend for lumefantrine. The data highlights the challenge of utilizing the CBEx when analyzing a fixed dose combination product where one API has a significantly lower concentration than another. It is important to note that these limitations are not exclusive to CBEx but intrinsic to most Raman spectrometers.

Both RHZE and RH samples are coated. The low match scores between spectra of the drug products as is and the sample powders and reference standards (see tables 8 and 9) indicates that the spectra of the products is not specific to any of the APIs. A method for analyzing RH or RHZE tablets would therefore need to be an analysis of the powder within the tablets rather than the tablet itself. The spectrum of RHZE and RH powder is most similar to RIR with match scores of 0.93 and 0.92, respectively and almost no agreement with any of the remaining three constituent APIs (ETR – 0.10, INR – 0.29, PYR – 0.12). There are however some characteristic PYR features in the RHZE spectrum, most likely due to its high concentration relative to the other components in the drug product. There is also good agreement between the RHZE and RH powders (0.88). Therefore, a substandard or falsified RHZE medicine without two or even three of the four APIs (isoniazid, pyrazinamide and ethambutol) may not be correctly identified using the CBEx.

Not even half of the AMX capsules (one half was pink and the other blue) provided API specific information. To obtain specific API information, analysis of amoxicillin capsules would need to be an analysis of the powder within the capsule rather than the capsule shell itself. However, there is good agreement between AMR and both AMX1 (0.99) and AMX2 (0.99), see table 10, indicates that the spectrum of the AMX samples is API specific and could be used to develop a reliable identification method.

Although acetaminophen characteristic peaks are apparent in the spectrum of both AC and AAC samples, there were significant matrix interferences, which resulted in low match scores – 0.56, 0.60 and 0.59 for AC1, AC2, and AAC, respectively. However, there was good agreement between the spectrum of AC1 and AC2, suggest that a non-brand specific drug product method could be developed. There was also good agreement between the AC1 capsule (which was translucent) and the AC1 liquid, indicating that the product could be analyzed non-destructively. Importantly, the spectrum of AC and AAC are significantly different, implying that an AC drug product would not pass as an AAC drug product.

There was good agreement between FSM1 and FSM2 (0.98) but almost no agreement between the two samples and FSR (0.20 and 0.22) most likely due to matrix effects. This indicates that an CBEx FSM drug product method would most likely not be specific to the API. Similar results were observed when analyzing oxytocin samples. Although there was good agreement between OXY1 and OXY2, this agreement seemed to be independent of the API. This is most likely due to the very small Raman cross section of oxytocin (see Annex 3, figure 13). SERS substrates were used to attempt to enhance the oxytocin Raman signal but the good agreement between a blank Au SERS substrate and the OXY1 incubated Au SERS substrate indicates that the substrates do not enhance the oxytocin Raman signal.

4.2. Field Evaluation

Based on feedback from trainees and the ongoing observations of the trainer, the training required to become a basic, intermediate or advanced user of the instrument was manageable. More specifically, a variety of staff with both technical and non-technical backgrounds can become either immediate or advanced users with less than 2 weeks of training. The PEAK software was easy to download onto an external computer through a vendor provided attachment and intuitive to use. Transfer of data and development of spectral libraries was also very simple with trainees developing libraries within a few minutes and analyzing samples within a few seconds at informal markets, pharmacies, wholesalers. The instrument is one of the smallest commercially available handheld Raman spectrometers and is self-contained, functioning with two AA batteries. Furthermore, it theoretically does not need any external consumables, making it particularly suitable for use in field settings where electricity may not be reliable.

Additional work would need to evaluate the feasibility of enhancing the instrument functionality to enable the development of spectral libraries directly from the instrument. Currently, spectral libraries can only be developed on an external computer. In standalone mode, the instrument does not display spectral match scores below the threshold of 0.85. Combined with the ability to develop libraries directly from the instrument, disabling this functionality to allow match scores of below 0.85 to be displayed would enhance the utility of the instrument, particularly in field settings.

References

[1] PQM, "Annual Performance Report FY 2017," Promoting the Quality of Medicines program, Washington, D.C., 2017.

[2] IOM, "Countering the Problem of Falsified and Substandard Drugs," Institute of Medicine (now the National Academies of Sciences, Engineering, Medicine), Washington, D.C., 2013.

[3] WHO, "WHO Model List of Essential Medicines, 20th List (March 2017)," March 2017b. [Online]. Available: http://www.who.int/medicines/publications/essentialmedicines/20th_EML2017.pdf?ua=1. [Accessed 10 December 2017].

[4] USP Review of Surveillance and Screening Technology for the Quality Assurance of Medicines Expert Panel, "Stimuli to the Revision Process: Evaluation of Screening Technologies for Assessing Medicine Quality (USP PF 43 (5))," September 2017. [Online]. Available: http://www.usppf.com/pf/pub/index.html. [Accessed 29 November 2017].

Annex 1. Equipment Used During Performance Evaluation

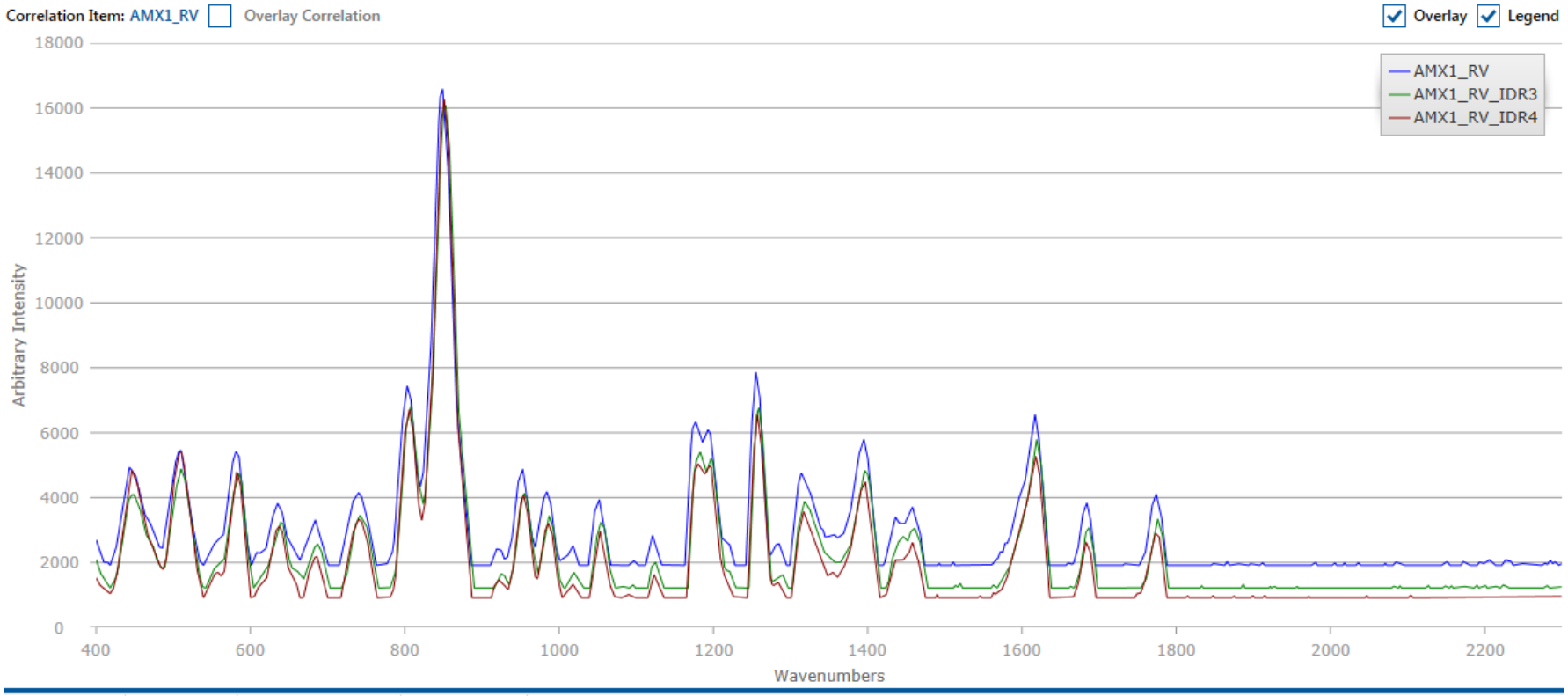
Item	Acronym	Manufacturer / Source	Expiry Date	Other details
High Performance Liquid Chromatography	HPLC	Agilent	N/A	Model: 1290 Infinity
CBEx – unit 1	CBEx2	Ocean Optics	N/A	Serial No: 2-0401-0785-051092
CBEx – unit 2	CBEx3	Ocean Optics	N/A	Serial No: 2-0401-0785-051093
CBEx – unit 3	CBEx4	Ocean Optics	N/A	Serial No: 2-0401-0785-051094
SERS Gold Nanoparticles	SERS Au	Diagnostic Ansers	N/A	Concentration: 0.35 mg/mL
SERS Silver Nanoparticles	SERS Ag	Diagnostic Ansers	N/A	Concentration: 0.11 mg/mL
Vacuum Oven	OV	Yamamoto Scientific	N/A	Model: ADP-21 Serial No: A3700054
Environmental Chamber	EC	Weisstechnik	N/A	Model: WKL 34/+10 Unit Not: 562460 10530010

Annex 2. Samples and Standards Used During Performance Evaluation

Item	Acronym	Manufacturer / Source	Lot Number	Expiry Date
Acetaminophen Reference Standard	ACR	USP	K2M244	N/A
Acetaminophen (325 mg) liquid capsules	AC1	CVS Health	1001568	Mar 2018
Acetaminophen (325 mg) liquid capsules	AC2	McNeil Consumer Healthcare	1440949	Sep 2018
Acetaminophen (250 mg) + Aspirin (250 mg) + Caffeine (65 mg) liquid capsules	AAC	CVS Health	P102707	Nov 2018
Amoxicillin Reference Standard	AMR	USP	L0K359	N/A
Amoxicillin (250 mg) capsules	AMX1	North Star x	AM2516029-B	Oct 2019
Amoxicillin (250 mg) capsules	AMX2	Aurobindo	AM2516016-A	Jul 2019
Artemether Reference Standard	ARR	USP	H0M313	N/A
Artemether (20 mg) + Lumefantrine (120 mg) tablets	AL1	Novartis	F0171W1	Feb 2018
Artemether (20 mg) + Lumefantrine (120 mg) tablets	AL2	Ipca Laboratories Ltd	DYI476161	Apr 2018
Artemether (20 mg) + Lumefantrine (120 mg) tablets	AL3	Ipca Laboratories Ltd	DYI466178	Apr 2018
Artemether (20 mg) + Lumefantrine (120 mg) tablets	AL4	Novartis	K0235	Jul 2018
Artemether (80 mg) + Lumefantrine (480 mg) tablets	AL5	Novartis	K0050	Oct 2018
Ethambutol Reference Standard	ETR	USP	H1J063	N/A
Furosemide Reference Standard	FSR	USP	M0M043	N/A
Furosemide injection	FSM1	Claris	A060212	Jan 2018
Furosemide injection	FSM2	Hospira Inc.	66-381-DK	Dec 2017
Isoniazid Reference Standard	ISR	USP	R013N0	N/A
Oxytocin Reference Standard	OXR	USP	F3K133	N/A
Oxytocin injection (10 units/mL)	OXY1	PAR Pharmaceuticals	300647	Jun 2018
Oxytocin injection (10 units/mL)	OXY2	APP Pharmaceuticals	6012568	Nov 2017
Pyrazinamide Reference Standard	PYR	USP	R030C0	N/A
Rifampin Reference Standard	RFR	USP	R039N0	N/A
Rifampicin (150 mg) + Isoniazid (150 mg) tablets	RH1	Phapros	6159001	Apr 2020
Rifampicin (!50 mg) + Isoniazid (150 mg) tablets	RH2	Macleods Pharmaceuticals Ltd.		
Rifampicin (150 mg) + Isoniazid (75 mg) + Pyrazinamide (400 mg) + Ethambutol HCl tablets (275 mg)	RHZE1	Macleods Pharmaceuticals Ltd.	HRT607A	Dec 2017
Rifampicin (150 mg) + Isoniazid (75 mg) + Pyrazinamide (400 mg) + Ethambutol HCl tablets (275 mg)	RHZE2	Kimia Farma	DE0880J	Apr-2018

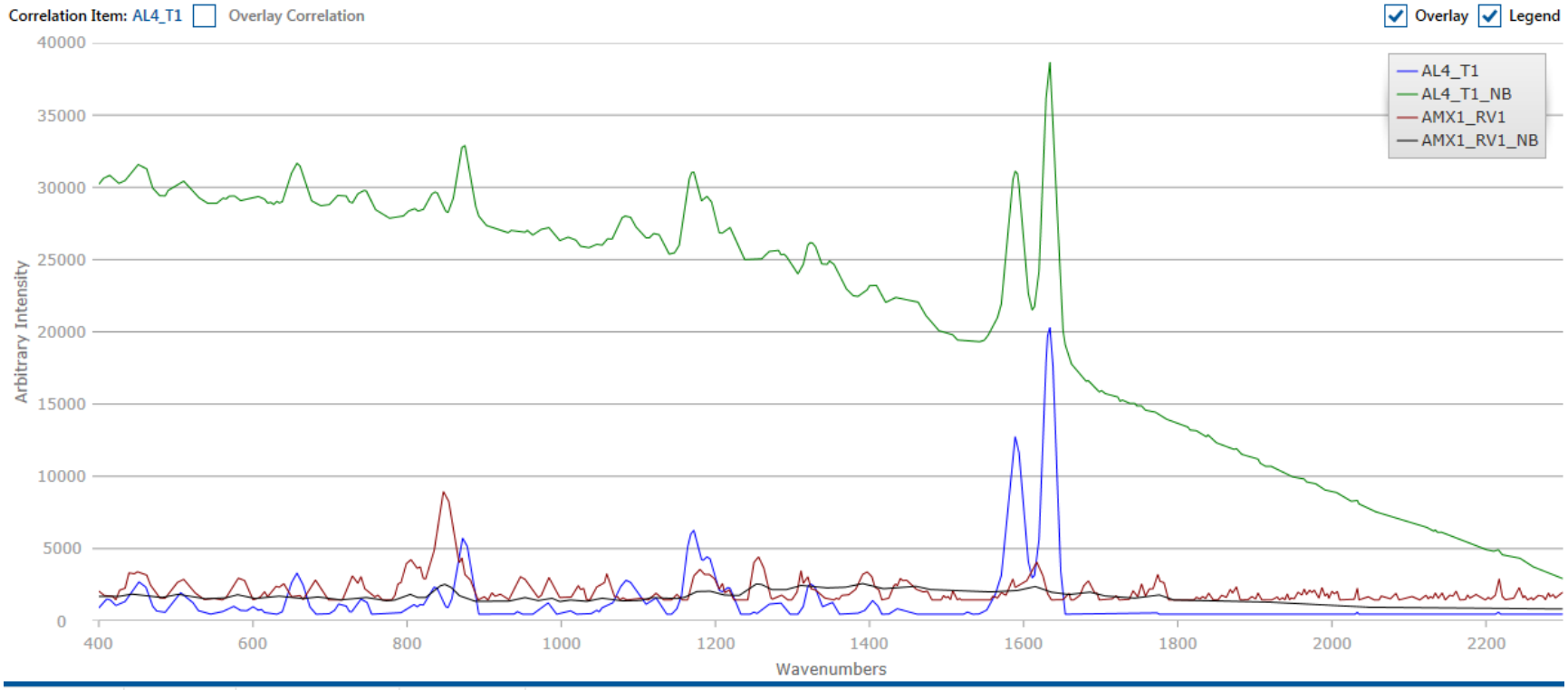
Annex 3. Spectral Overlay Figures for Various Comparisons

Figure 5. Instrument Reproducibility – Spectra Comparison for Amoxicillin Capsules Powder (AMX1) from CBEx2, CBEx3 and CBEx4 using CBEx2 as the Reference Spectrum



Name	MatchScore	SerialNum	SourceLibrary	Path
AMX1_RV	1.00	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\Amoxicillin spectra\AMX1_RV.rmn
AMX1_RV_IDR3	0.94	2-0401-0785-051093	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\Amoxicillin spectra\AMX1_RV_IDR3.rmn
AMX1_RV_IDR4	0.97	2-0401-0785-051094	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\Amoxicillin spectra\AMX1_RV_IDR4.rmn

Figure 6. Comparison of Baselined and Non-Baselined Artemether + Lumefantrine Tablets (AL4) and Amoxicillin Capsules (AMX1) Spectra



Name	MatchScore	SerialNum	SourceLibrary	Path
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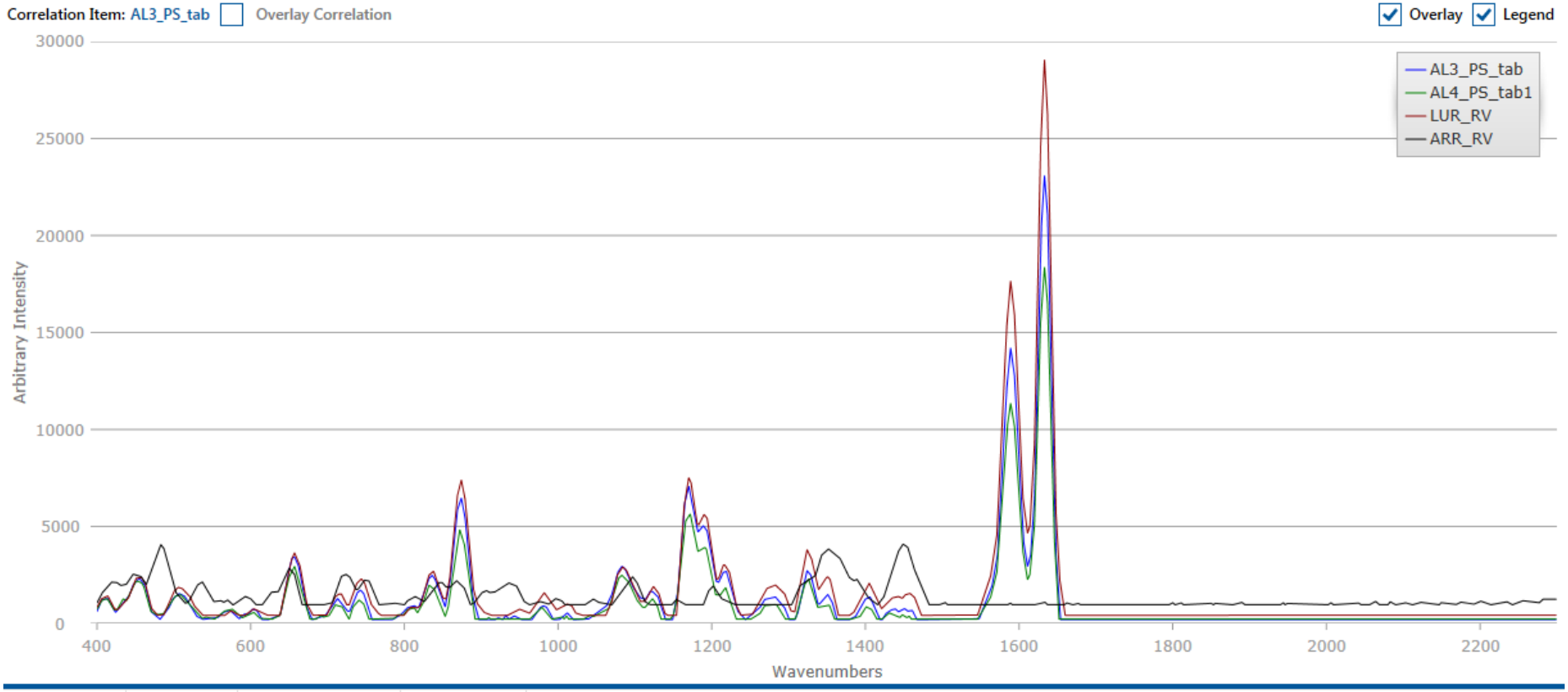
AL4_T1_NB	0.14	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_Background and Raster\AL4_T1_NB.rmn
AMX1_RV1	0.03	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_Background and Raster\AMX1_RV1_B.rmn
AMX1_RV1_NB	0.07	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_Background and Raster\AMX1_RV1_NB.rmn

Figure 7. Spectra Comparison of Artemether 20 mg + Lumefantrine 120 mg Tablets (AL3 and AL4), Artemether RS (ARR), and Lumefantrine RS (LUR) using AL3 as the Reference Spectrum



Name	MatchScore	SerialNum	SourceLibrary	Path
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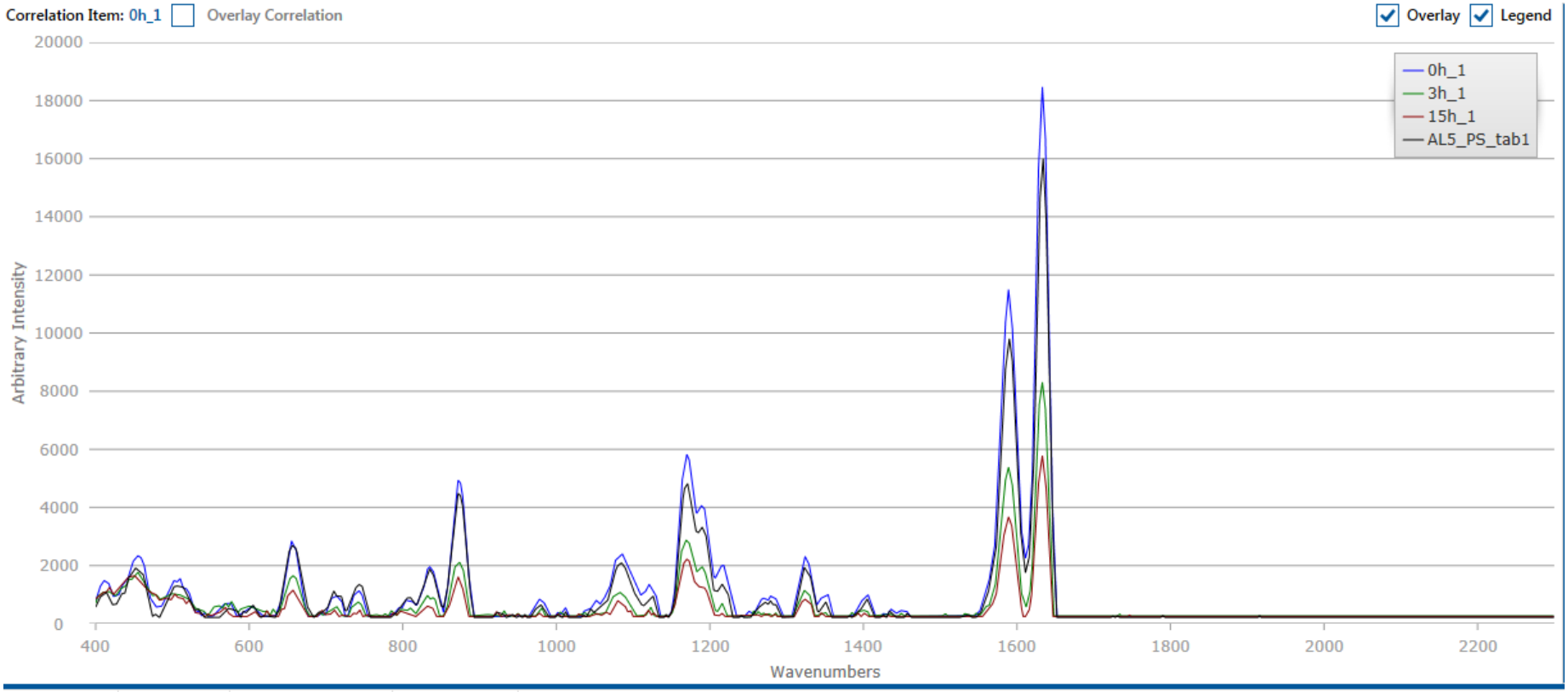
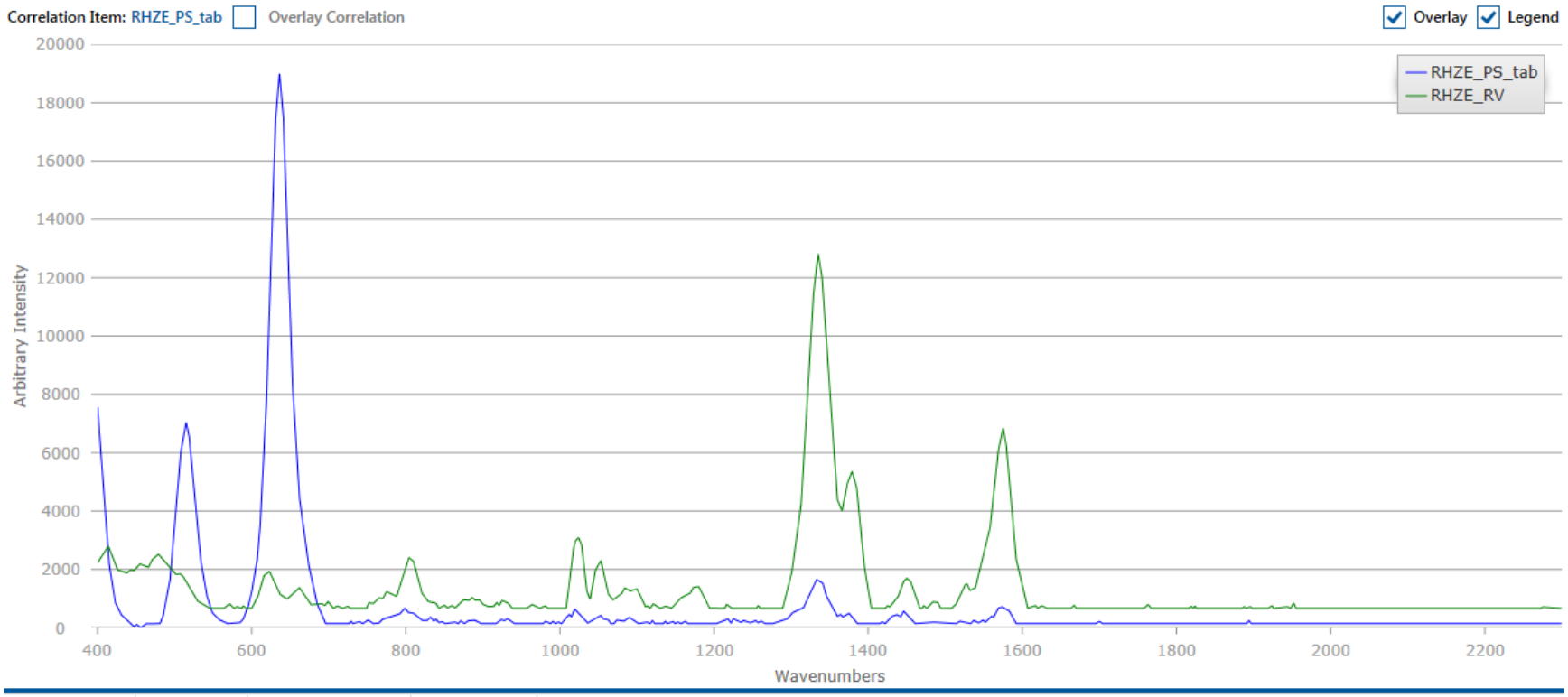
AL4_PS_tab1	0.99	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_AL Brand, Preparation and RS Comparison\Al
ARR_RV	0.00	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_AL Brand, Preparation and RS Comparison\Al
LUR_RV	0.99	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_AL Brand, Preparation and RS Comparison\LU

Figure 8. Spectra Comparison for Three Degraded Conditions for Artemether 20 mg + Lumefantrine 120 mg Tablets (AL3) and Non-Degraded Artemether 80 mg + Lumefantrine 480 mg Tablets (AL5)



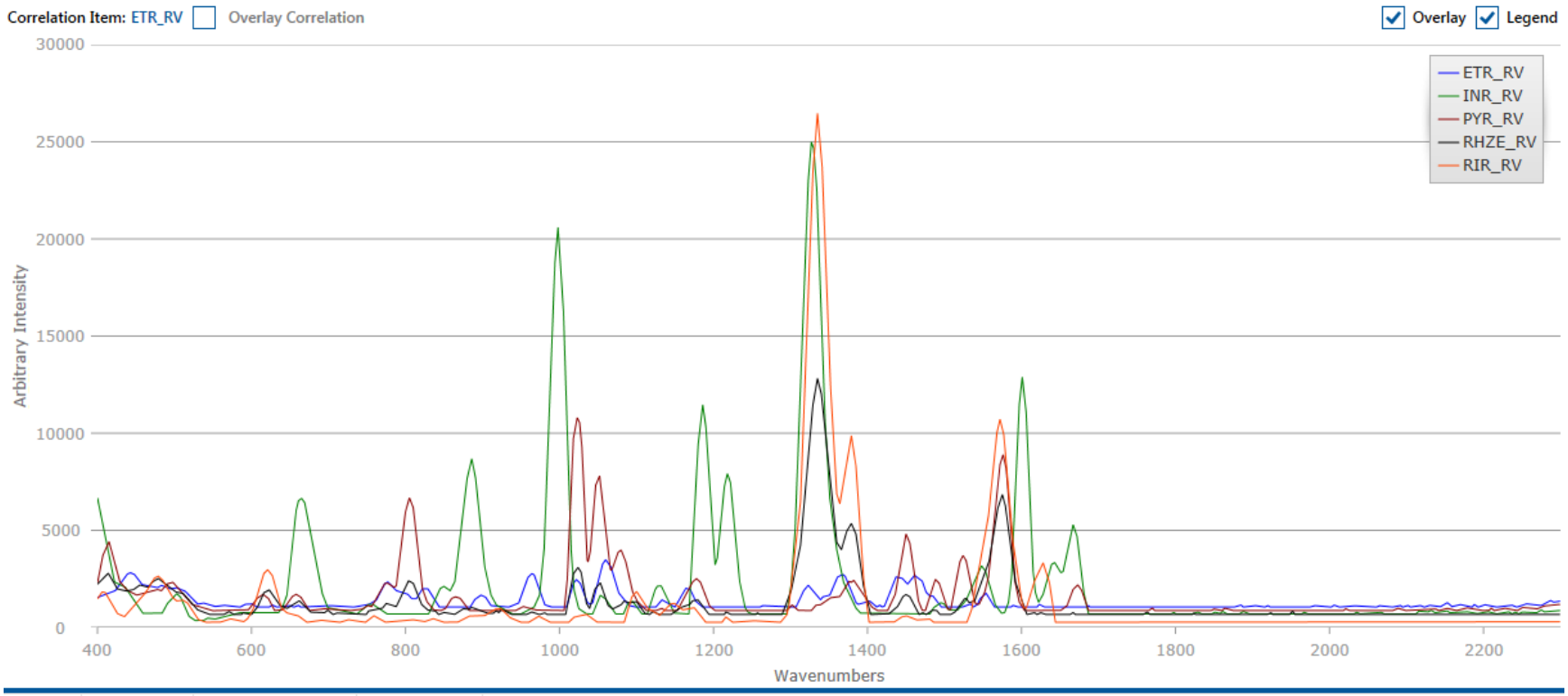
Name	MatchScore	SerialNum	SourceLibrary	Path
0h_1	1.00	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_AL Degradation and Dosage Strength Compar
3h_1	0.96	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_AL Degradation and Dosage Strength Compar
15h_1	0.90	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_AL Degradation and Dosage Strength Compar
AL5_PS_tab1	0.99	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_AL Degradation and Dosage Strength Compar

Figure 9. Spectra Comparison of Rifampicin 150 mg + Isoniazid 75 mg + Pyrazinamide 400 mg + Ethambutol 275 mg Tablets Powder and Tablet as Is (RHZE1)



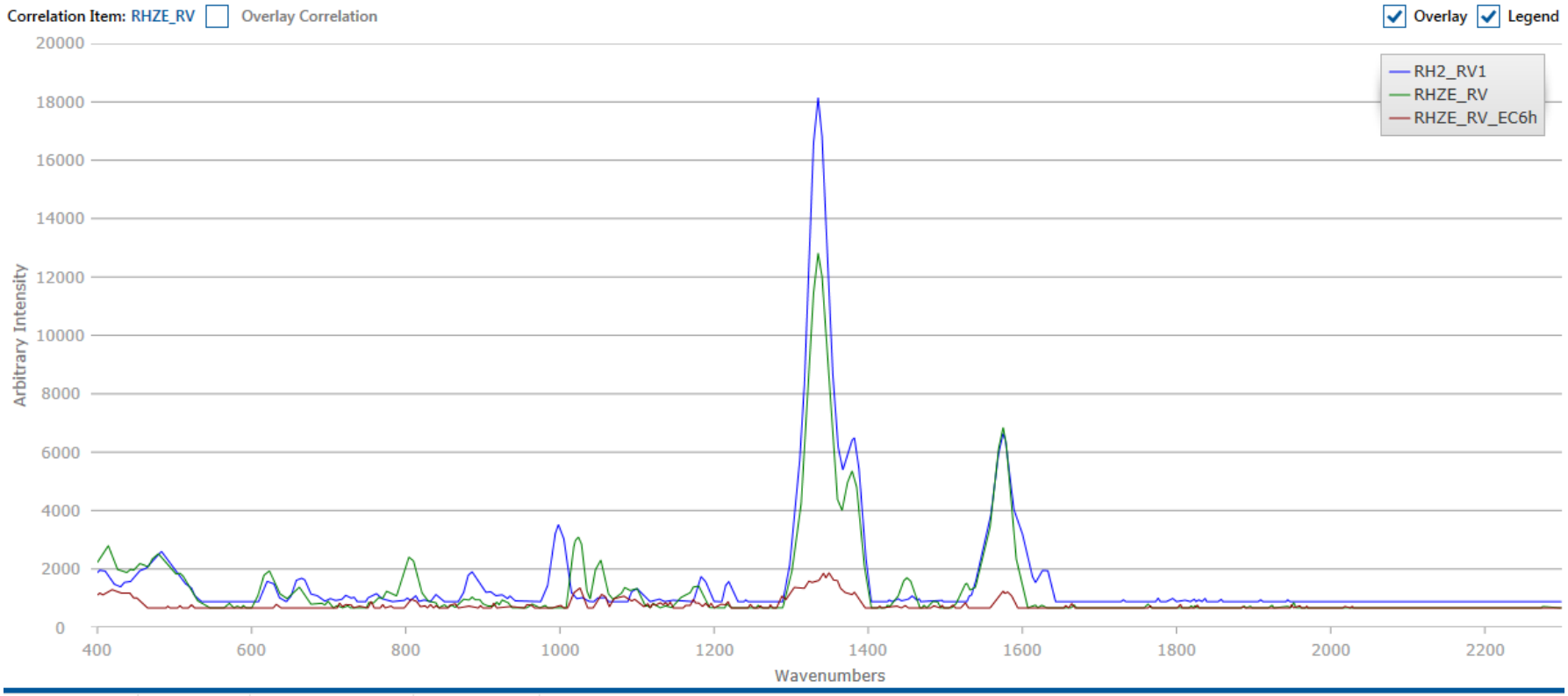
Name	MatchScore	SerialNum	SourceLibrary	Path
RHZE_PS_tab	1.00	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_RHZE Brand, Preparation and RS Comparis
RHZE_RV	0.01	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_RHZE Brand, Preparation and RS Comparis

Figure 10. Spectra Comparison of Rifampicin 150 mg + Isoniazid 75 mg + Pyrazinamide 400 mg + Ethambutol 275 mg Tablets Powder (RHZE1) and the Four Constituent APIs – Ethambutol (ETR), Isoniazid (INR), Pyrazinamide (PYR), and Rifampicin (RIR)



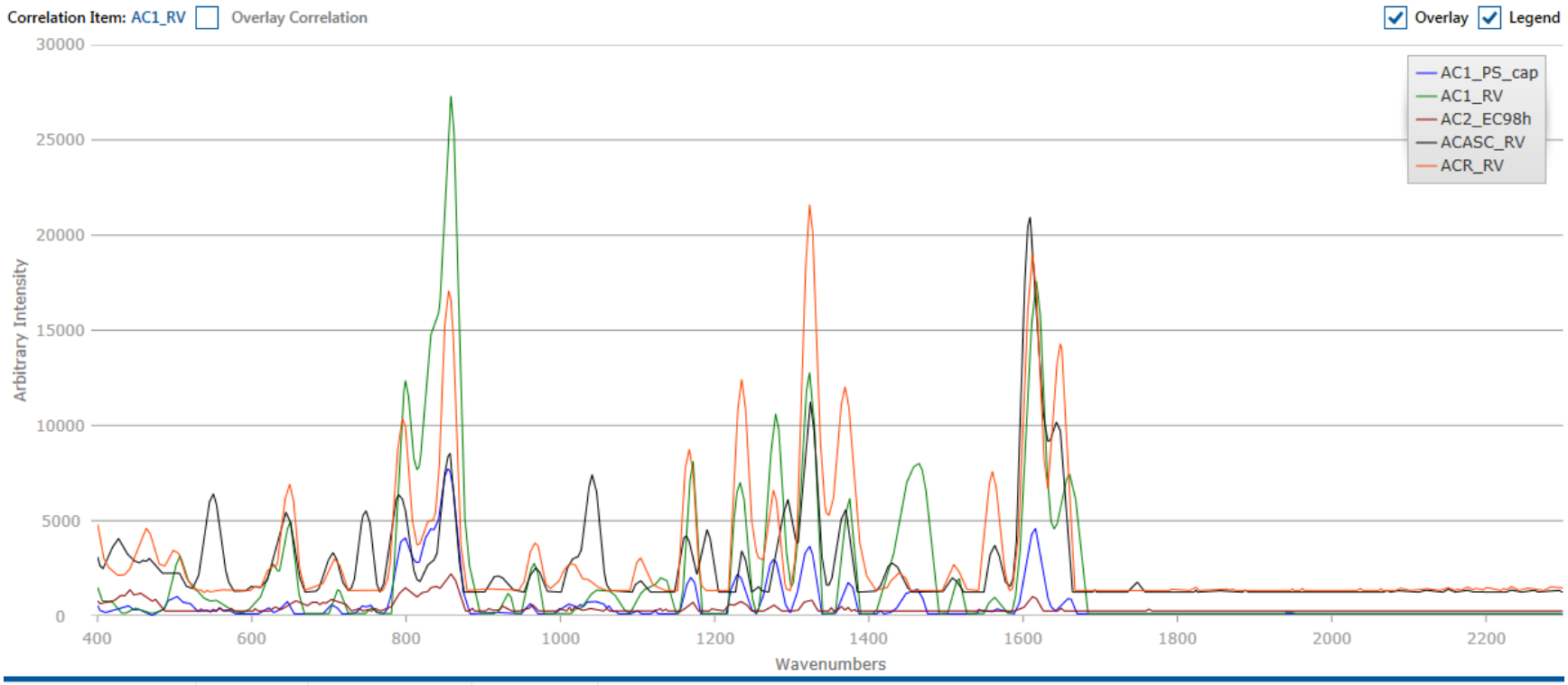
Name	MatchScore	SerialNum	SourceLibrary	Path
ETR_RV	1.00	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_RHZE Brand, Preparation and RS Comparison\ETR_
INR_RV	0.00	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_RHZE Brand, Preparation and RS Comparison\INR_
PYR_RV	0.18	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_RHZE Brand, Preparation and RS Comparison\PYR_
RHZE_RV	0.10	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_RHZE Brand, Preparation and RS Comparison\RHZE
RIR_RV	0.03	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_RHZE Brand, Preparation and RS Comparison\RIR_

Figure 11. Spectra Comparison of Rifampicin 150 mg + Isoniazid 75 mg + Pyrazinamide 400 mg + Ethambutol 275 mg Tablets Powder (RHZE1), RHZE1 Exposed Powder, and Rifampicin 150 mg + Isoniazid 150 mg Tablets Powder (RH2)



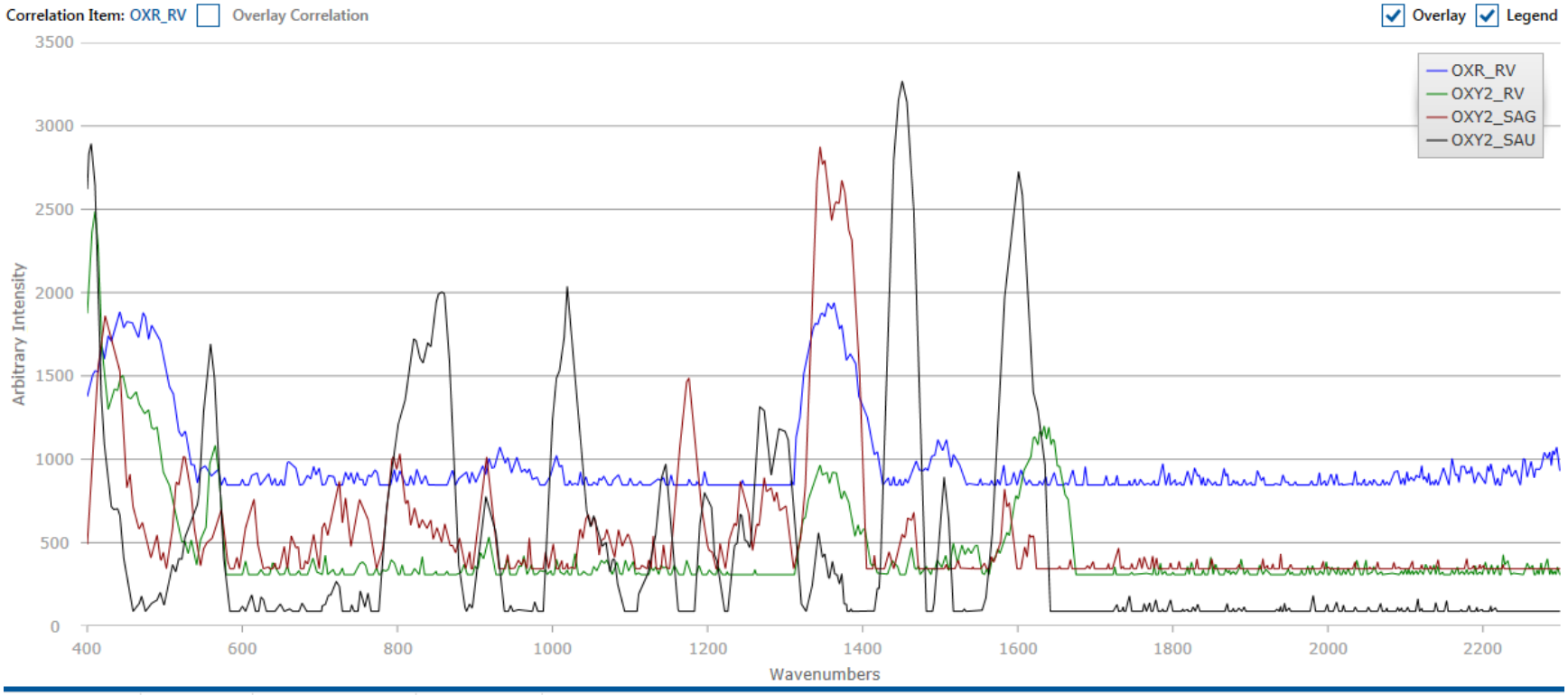
Name	MatchScore	SerialNum	SourceLibrary	Path
RH2_RV1	0.91	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_RHZE Degradation and Fixed Dose Combir
RHZE_RV	1.00	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_RHZE Degradation and Fixed Dose Combir
RHZE_RV_EC6h	0.67	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_RHZE Degradation and Fixed Dose Combir

Figure 12. Spectra Comparison of Acetaminophen 325 mg Capsules (AC1), AC1 Liquid, Degraded Acetaminophen 325 mg Capsule Liquid (AC2), Acetaminophen 250 mg + Aspirin 250 mg + Caffeine 65 mg Capsule Liquid (AAC), and Acetaminophen Reference Standard (ACR)



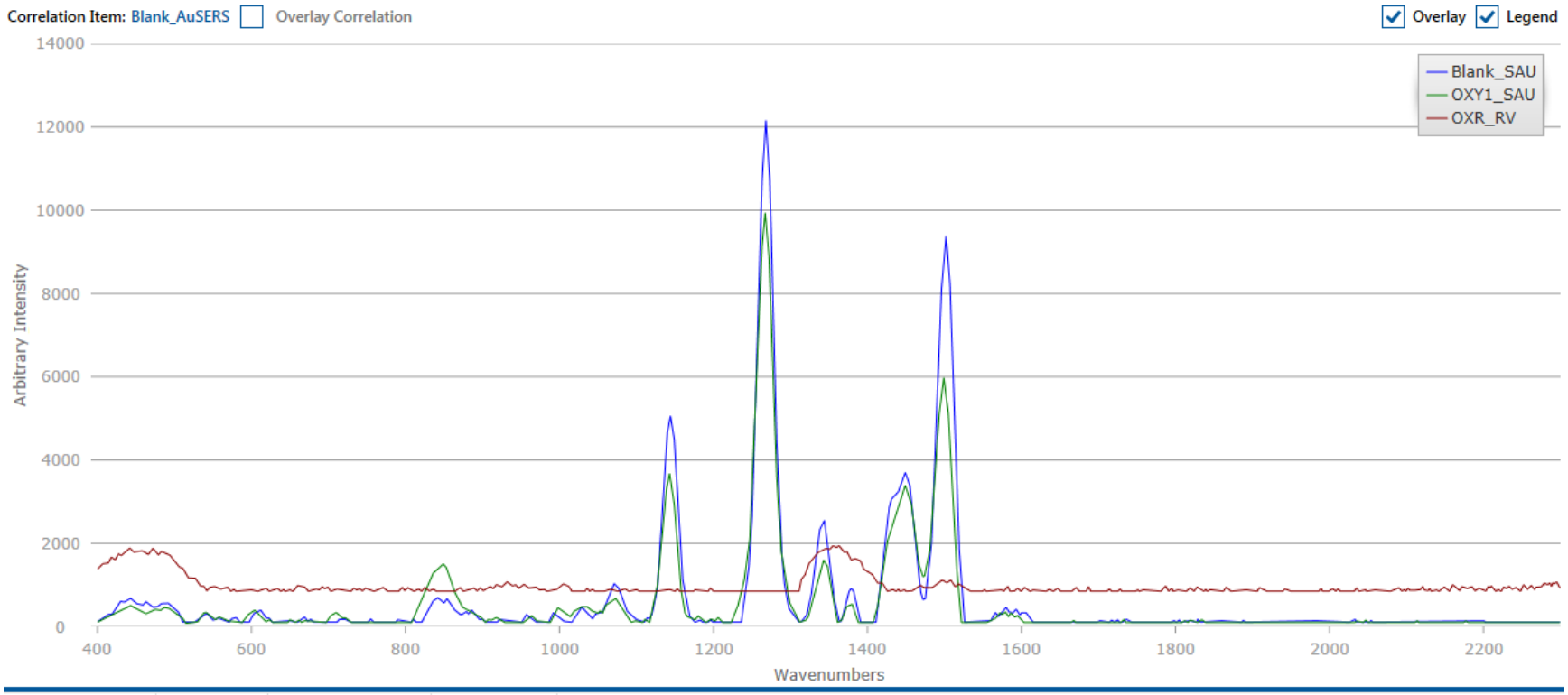
Name	MatchScore	SerialNum	SourceLibrary	Path
AC1_PS_cap	0.89	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_AC Brand, Degradation, Preparatic
AC1_RV	1.00	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_AC Brand, Degradation, Preparatic
AC2_EC98h	0.47	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_AC Brand, Degradation, Preparatic
ACASC_RV	0.34	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_AC Brand, Degradation, Preparatic
ACR_RV	0.56	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_AC Brand, Degradation, Preparatic

Figure 13. Spectra Comparison of Oxytocin Reference Standard (OXR), Oxytocin 10 units/mL Injection (OXY2) as is, and OXY2 on the Silver (Ag) and Gold (Au) SERS Substrates



Name	MatchScore	SerialNum	SourceLibrary	Path
OXR_RV	1.00	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_OXY and FSM Brand, Degradation, Prepara
OXY2_RV	0.00	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_OXY and FSM Brand, Degradation, Prepara
OXY2_SAG	0.48	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_OXY and FSM Brand, Degradation, Prepara
OXY2_SAU	0.00	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_OXY and FSM Brand, Degradation, Prepara

Figure 14. Spectra Comparison Oxytocin Reference Standard (OXR), a Blank SERS Gold (Au) Substrate, and a SERS Au Substrate Incubated with Oxytocin 10 units/mL Injection (OXY1)



Name	MatchScore	SerialNum	SourceLibrary	Path
Blank_SAU	1.00	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\SERS\Blank_AuSERS.rmn
OXY1_SAU	0.95	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\SERS\OXY1_AuSERS_drop.rmn
OXR_RV	0.00	2-0401-0785-051092	N/A	C:\Users\LMR\Desktop\Desktop\TR\ID Raman mini 2.0 (Ocean Optics)\Data\IDRaman Data\TR Report_OXY and FSM Brand, Degradation, Prepa