Tuberculosis medications and non-destructive compliance screening with comparison of handheld and benchtop diffuse reflectance spectrometers

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BACKGROUND

Tuberculosis is one of the world's leading causes of death. Medications such as isoniazid have a long track record of treating Tuberculosis infections efficiently, but the quality and efficacy of these medications must be ensured. Benchtop diffuse reflectance spectroscopy (DRS) has a history of screening finished pharmaceutical products (FPP)s. As technologies have advanced, so have handheld DRS (HHDRS), in terms of portability and cost, offering potential for screening FPPs in localized settings. However, careful consideration to what the HHDRs is measuring must be considered. Here, comparison of several TB treatment medications are compared with a benchtop DRS and a HHDRS.

METHOD

Spectrometers

A benchtop spectrometer (Labspec 5000, Malvern Panalytical, Malvern, UK) collected spectra every 1 nm between 350 – 2500 nm, while a handheld spectrometer collected spectra at a variable 2 – 4 nm between 900 – 1700 nm (**Fig. 1**).

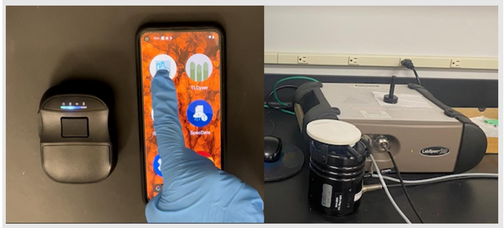

FIGURE 1. **(L): Handheld DRS paired with Smartphone, (R): Benchtop DRS**

Tablets

Medications were obtained through a global donation-based supply chain. Four products containing active pharmaceutical ingredients (API)s can be seen in **Table 1**. Tablets contain either a) isoniazid, b) rifapentine, c) isoniazid & rifapentine, or d) isoniazid, pyridoxine, sulfamethoxazole, & trimethoprim. Additionally, tablets were pressed in-house containing 100% isoniazid as a reference. Tablets were scanned in triplicate with the mean spectra calculated per tablet and used in analyses.

TABLE 1. **Active pharmaceutical ingredients of tuberculosis medications scanned with spectrometers**

Active Ingredients	Formula	# Tablets
Isoniazid (Control)	$C_6H_7N_3O$	12
Isoniazid	$C_6H_7N_3O$	110
Rifapentine	$C_{47}H_{64}N_4O_{12}$	150
Isoniazid & Rifapentine	$C_6H_7N_3O$ $C_{47}H_{64}N_4O_{12}$	140
Isoniazid Pyridoxine Sulfamethoxazole Trimethoprim	$C_6H_7N_3O$ $C_8H_{11}NO_3$ $C_{10}H_{11}N_3O_3S$ $C_{14}H_{18}N_4O_3$	150
Total		*562*

Data Analysis

Spectra were exported as either TXT (benchtop) or CSV (handheld) files and analyzed with R-Studio (v.4.2), using *"2DCorr", "MCR", and "Chemometrics"* packages.

RESULTS

Spectra

Spectra were preprocessed with a standard normal variant (SNV), and mean spectra for each product are shown below in **Fig. 2.**

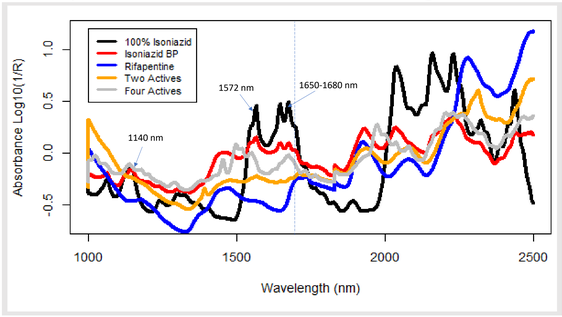

FIGURE 2. **Preprocessed (SNV) mean spectra of common TB medications and isoniazid control. Dashed vertical line is limit of HHDRS range.**

Plotting out the preprocessed mean spectra shows that there are relevant spectral peaks outside of the range of the HHDRS, but between 900 – 1700 the benchtop DRS detects several isoniazid related peaks that are not seen in the rifapentine spectra at 1140, 1572, and 1650 – 1680 nm. Peaks are present in the pure isoniazid reference, isoniazid BP, and both the two-API, and four-API tablets.

2D Correlation Spectroscopy

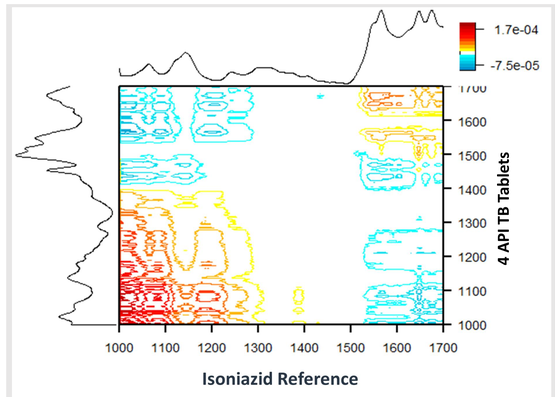

FIGURE 3. **2D Correlation plot comparing Isoniazid control and the 4 API product.**

The 2D correlation map in **Fig. 3** shows that there are regions within the collection range of the HHDRS (900 – 1700 nm) from the 4 active ingredient TB tablets to the reference isoniazid at approximately 1050 and 1650 - 1680 nm. There are other noticeable isoniazid correlations that are outside of the range of the HHDRS.

Multivariate Curve Resolution (MCR)

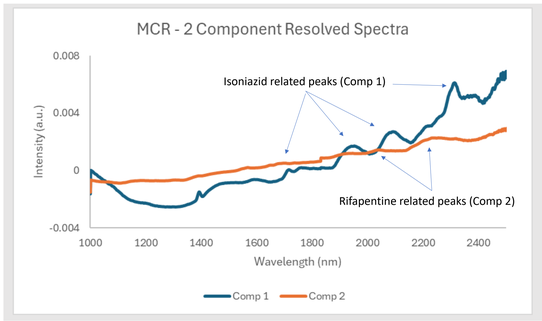

FIGURE 4. **Multivariate Curve Resolution – resolved component spectra of isoniazid from the 2 API product.**

Resolved spectra from the MCR (**Fig. 4**) showed that the 1st component closely resembles isoniazid spectra, while peaks from the rifapentine can be seen in component 2. Most of these characteristic peaks do appear outside the range of the HHDRS, suggesting that MCR may not be ideal for analyzing the HHDRS spectra, but performs well on the benchtop.

TABLE 2. **Isoniazid related peaks and their presence in the HHDRS range (900 – 1700 nm).**

Active	Wavelength (nm)	TB Medication	Approximate Assignment[1]
Isoniazid	~ 1030	Iso Ref, Iso BP, 4-API	3rd Overtone RNH_2
Isoniazid	~ 1140	Iso Ref, Iso BP, 4-API	2nd Overtone CH_2/CH_3
Isoniazid	~ 1572	Iso. Ref, Iso. BP, 2-API, 4-API	1st overtone
Isoniazid	~1650 - 1680	Iso. Ref, Iso. BP, 2-API, 4-API	1st overtone CH_3

Iso. Ref. = Isoniazid reference tablets, Iso. BP = Isoniazid BP

Several key spectral peaks (**Table 2**) associated with isoniazid were found in the mean spectra within the range of the HHDRS and were confirmed through 2D Correlation and MCR as being present in the multi-active TB tablets. Rifapentine, another important API in TB medications had one strong peak present in the range of the HHDRS but is commonly associated with moisture at approximately 1440 nm. Prominent spectral peaks associated with rifapentine were found > 1700 nm with the benchtop spectrometer.

Conclusions

- HHDRS doesn't capture as many isoniazid related spectral peaks as the benchtop but does capture key isoniazid peaks in the reference and multi-API tablets.
- Isoniazid peaks were differentiated from other APIs in multi-API tablets due to CH_2/CH_3 and RNH_2 bonds at 1140, 1650-1680, and 1030 nm.[1]
- HHDRS is a more affordable and portable option for screening TB medications for isoniazid presence, with potential future field applications.
- Rifapentine may have difficulty in qualitative screening due to majority of corresponding peaks located > 1700 nm.

References

[1]NIR Spectroscopy: A guide to near-infrared spectroscopic analysis of industrial manufacturing. 2013. Metrohm. Herisau, Switzerland.

Acknowledgments

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