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Development and Validation of a Liquid Chromatography-Tandem Mass Spectrometry Method for Quantifying Delamanid and its Metabolite in Small Hair Samples

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Abstract

New all-oral regimens for rifampin-resistant tuberculosis (RR-TB) are being scaled up globally. Measurement of drug concentrations in hair assesses long-term drug exposure. Delamanid (DLM) is likely to be a key component of future RR-TB treatment regimens, but a method to describe its quantification in hair via liquid chromatography-tandem mass spectrometry (LC-MS/MS) has not previously been described. We developed and validated a simple, fast, sensitive, and accurate LC-

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Ali Esmail-resources-sample collection and curation

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Conflict of Interest

All authors declare no conflict of interest in the conduct of the work described in the manuscript being submitted.

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MS/MS method for quantifying DLM and its metabolite DM-6705 in small hair samples. We pulverized and extracted two milligrams of hair in methanol at 37°C for two hours, and diluted 1:1 with water. A gradient elution method eluted DLM, DM-6705, and the internal standard OPC 14714 within 3 minutes, bringing overall analysis time to 5.5 minutes. The method has limits of detection (LOD) of 0.0003 ng/mg for DLM and 0.003 ng/mg for DM-6705. The established linear dynamic ranges are 0.003–2.1 ng/mg and 0.03–21 ng/mg for DLM and DM-6705, respectively. Eleven of 12 participant hair samples had concentrations within DLM's linear dynamic range, while all 12 samples had concentrations within the quantifiable range for DM-6705. The ranges of concentrations observed in these clinical samples for DLM and DM-6705 were 0.004–0.264 ng/mg hair and 0.412–12.041 ng/mg hair respectively. We demonstrate that while DLM was detected in hair at very low levels, its primary metabolite DM-6705 had levels approximately 100 times higher. Measuring DM-6705 in hair may accurately reflect long-term adherence to DLM-containing regimens for drug-resistant TB.

Keywords

LC-MS/MS; hair analysis; drug-resistant tuberculosis; objective adherence metrics; delamanid; DM-6705

1. Introduction

Rifampin-resistant tuberculosis (RR-TB) remains a global public health crisis with over 558,000 incident cases per year [1]. Subtherapeutic concentrations of anti-TB drugs (in either drug sensitive or RR-TB) are common [2–4], in particular among people living with HIV [5–6], and have been associated with acquired drug resistance [7–10] and adverse treatment outcomes in drug-sensitive TB [11–12]. Measuring drug levels of anti-TB drugs as objective pharmacologic measures of adherence may critically inform RR-TB management and clinical trials. Unlike HIV regimens where a combination of drugs in a single pill is common, TB medications are taken separately, so adherence monitoring must be performed for every single drug in the regimen. Drug levels in plasma are most commonly measured, but these require phlebotomy, cold chain, and specialized equipment. Hair levels of drugs have been used as a long-term metric of adherence in HIV therapy [13]. Small hair samples are easily collected can be stored at room temperature, and levels in hair are minimally affected by short-term spikes in adherence [14].

Delamanid (DLM, OP 67683, Deltyba), a bicyclic nitroimidazole that exerts activity against M. tuberculosis through generation of intracellular nitric oxide, first demonstrated preclinical efficacy in 2006 [15] and exposure-dependent early bactericidal activity in 2010 [16]. Decreased mortality and increased two-month sputum-culture conversion was noted in a phase IIb trial [17], though a subsequent phase III trial (Trial 213) failed to demonstrate increased treatment success or shorter time to culture conversion when DLM was added to an optimized background regimen [18]. Nevertheless, DLM was approved as an option for RR-TB in 2018 since there was a dire need for RR-TB treatment options. Moreover, DLM has limited drug-drug interactions with antiretroviral medications and may effectively replace or protect other medicines in RR-TB regimens [19]. Therefore, along with

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bedaquiline and pretomanid, another nitroimidazole, DLM is likely to become an important anti-TB medication for RR-TB globally with scale-up being monitored closely [20].

DLM is a prodrug and is extensively metabolized to at least eight metabolites via a number of pathways [21]. LC-MS/MS methods to measure DLM [15,22,23] and its major metabolite, DM-6705 [15,22], in serum and plasma have been developed. We describe here the development and validation of the first LC-MS/MS method for the quantitative analysis of DLM and its major metabolite DM-6705 in small hair samples. This study extends our previous work developing a multi-analyte panel for other RR-TB drugs in small hair samples for adherence and exposure monitoring [24–27].

2. Materials and Methods

2.1 Chemicals

We purchased reference standards for DLM (>99% chemical purity) from MedKoo Biosciences and the internal standard, OPC 14714 (98% chemical purity), from Toronto Research Chemicals. DM-6705 was donated by Otsuka Pharmaceutical Co. Ltd. (Tokyo, Japan). We purchased HPLC-grade water from Aqua Solutions, Inc. and HPLC-grade acetonitrile and methanol from Honeywell Burdick and Jackson. We purchased > 95% purity formic acid from Sigma-Aldrich.

2.2 Sample Analysis

For analyte extraction, we cut hair strands to 3 cm and weighed out 2 mg on a Sartorius CP124S with 0.1 mg accuracy. We then pulverized the hair using an Omni Bead Ruptor homogenizer. We prepared a solution of 16 ng/mL OPC 14714 in methanol and added 500 μL of that mix to each tube, followed by incubation at 37°C for two hours. After incubation, we added 500 μL water, vortexed, centrifuged, and used the supernatant for LC-MS/MS analysis.

For analysis, we used an LC-MS/MS system (Agilent LC 1260-AB Sciex API 5500, Agilent Technologies, and AB Sciex). We injected 7 μL of sample extract into a Synergi Polar RP column (2.1 × 100 mm, 2.5 μm particle size, Phenomenex) using a gradient elution of water with 0.1% formic acid as mobile phase A (MPA) and acetonitrile as mobile phase B (MPB). The gradient consisted of 65% to 75% MPB from 0–1.68 min, gradient to 100% MPB from 1.68–2.4 min, 100% MPB from 2.4–3.3 min, gradient to 65% MPB from 3.3–3.4 min, and 65% MPB from 3.4–5.5 min. We used electrospray ionization in positive polarity mode and multiple reaction monitoring to scan for masses of the analytes.

We injected quality control (QC) samples at low (0.018 ng/mg DLM, 0.180 ng/mg DM-6705), mid (0.180 ng/mg DLM, 1.8 ng/mg DM-6705), and high (1.8 ng/mg DLM, 18 ng/mg DM-6705) levels and a method blank alongside each calibration curve. These were run at the beginning, middle, and end of each run. QC preparations passed if they were within 15% of the target value and their precision within 15% coefficient of variation (CV).

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2.3 Data Analysis

We used AB Sciex Analyst 1.6 and AB Sciex MultiQuant 2.1 for data analysis. We confirmed peak identifications for each analyte using two transitions and retention time. Both DLM and DM-6705 were quantified using area of the drug over area of OPC 14714, which was added to each vial at a total concentration of 8 ng/mL. We used linear regression with 1/x weighting to form the calibration curve.

2.4 Method Validation

We validated the method's linearity, specificity, sensitivity, precision, accuracy, matrix effect, recovery, injection repeatability, and carryover. To test linearity, we prepared and ran a calibration curve (0.0003–2.1 ng/mg hair for DLM, 0.003–21 ng/mg hair for DM-6705) three separate times across three days and checked for a passing (>95%) coefficient of determination each time. To test specificity, we used hair samples from six RR-TB patients who had not taken DLM. We defined the limit of detection (LOD) as the lowest concentration in our series of calibration standards that had a signal to noise ratio ≥3. We defined the lower limit of quantitation (LLOQ) as the lowest concentration that had a signal to noise ratio ≥10, while keeping the coefficient of determination >95%.

To assess precision and accuracy, we spiked blank hair samples at the LLOQ and with low, mid, and high QC concentrations of the analytes. We ran five replicates of each of the four concentration levels and prepared them separately for three runs over three days. The precision and accuracy pass if the CV and relative error (RE) are ≤ 15% at the QC levels and ≤ 20% at the LLOQ. For testing matrix effect, we spiked five replicates of the three concentrations of the analytes into 1:1 methanol:water and compared it to the average area when spiked into extracted matrix. We subtracted the average area of the solvent replicates from the average area of the matrix replicates and then divided it by the average area of the solvent replicates to determine matrix effect percentage. For testing recovery, we spiked five replicates of the three concentrations of the analytes into extracted matrix and compared it to the average area when analytes were spiked into matrix and then extracted. We divided the average area of the replicates spiked prior to extraction by the average area of the replicates spiked following extraction to determine recovery percentage.

We tested injection repeatability by injecting the analytes at the three concentrations five times in one day. We tested carryover by injecting the analytes at high concentration one time, then two times, then three times, each time preceded and followed by three matrix blanks.

2.5 Patient Hair Testing

Between 1 July 2016 and 1 Jan 2018, small hair samples (20–50 strands) were collected from 12 patients undergoing inpatient, directly observed treatment for RR-TB at the Brooklyn Chest Hospital, Cape Town, South Africa. Each participant provided written informed consent, and ethical approval was obtained from the University of Cape Town Human Research Ethics Committee (187/2016) and the University of California, San Francisco Human Research Protection Program.

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Hair samples from the participants were analyzed using our validated method. All participants received a total of 200 mg delamanid daily. We used the 3 cm of participant hair closest to the scalp, which would grow over the course of 90 days on average [28]. If participants had hair ≤ 3 cm, we used the whole strand. However, five of the 12 participants had treatment durations shorter than 90 days. Hence, we corrected for the lack of drug in segments of these hair samples by dividing the measured concentration of DLM and DM-6705 by the ratio of number of treatment days over total days of hair growth (90 days). Correlations between measured DLM and DM-6705 concentrations and duration of treatment were assessed using linear regression and rank correlation analyses. None of the hair samples had visible signs of dye or perm treatment, as all were curly and black.

3. Results

3.1 Method Development

We developed a 5.5-minute method to quantitatively analyze DLM and its metabolite DM-6705 in small (2 mg) hair samples using LC-MS/MS. For the two target analytes and the chosen internal standard (OPC 14714), we analyzed two mass spectral transitions (Figure 1). We used the most abundant transition as the quantifier ion and the other transition as the qualifier ion (Table 1).

3.2 Method Validation

3.2.1 Specificity—Neither of the analytes were detected in the hair samples of the six control RR-TB patients.

3.2.2 Linearity and Sensitivity—The LOD was 0.0003 ng/mg hair for DLM and 0.003 ng/mg hair for DM-6705. For DLM, the LLOQ was 0.003 ng/mg hair and the upper limit of quantitation (ULOQ) was 2.1 ng/mg hair. For DM-6705, the LLOQ was 0.03 ng/mg hair and the ULOQ was 21 ng/mg hair. The average linearity coefficients of determination for DLM and DM-6705 were 0.997 and 0.995 respectively (Supplementary Table).

3.2.3 Precision and Accuracy—For DLM, the within-run precision at the low, mid, and high QC levels was 4.24%, 2.87%, and 4.06% respectively. At the low, mid, and high QC levels, the between-run precision was 8.64%, 8.40%, and 7.61% CV respectively. At the LLOQ, within-run precision was 2.34% CV and between-run precision was 2.53% CV. For DM-6705, within-run precision at the low, mid, and high QC levels was 2.10%, 1.32%, and 3.12% respectively. At the low, mid, and high QC levels, the between-run precision was 3.30%, 2.11%, and 5.36% CV respectively. At the LLOQ, within-run precision was 3.81% CV and between-run precision was 3.47% CV. (Table 2).

For DLM, the within-run accuracy at the low, mid, and high QC levels was 8.89%, 5.56%, and 5.30% respectively. At the low, mid, and high QC levels, the between-run accuracy was 8.01%, 7.94%, and 8.92% RE respectively. At the LLOQ, within-run accuracy was 15.64% RE and between-run accuracy was 11.82% RE. For DM-6705, the within-run accuracy at the the low, mid, and high QC levels was 5.74%, 11.04%, and 4.59% respectively. At the low, mid, and high QC levels, the between-run accuracy was 9.00%, 12.94%, and 5.88% RE

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respectively At the LLOQ, within-run accuracy was 9.45% RE and between-run accuracy was 8.44% RE (Table 2).

3.2.4 Matrix Effect and Recovery—The matrix effect of DLM at the low, mid, and high QC levels was −0.03%, −4.02%, and −7.86% respectively. The matrix effect of DM-6705 at the low, mid, and high QC levels was 2.04%, 2.18%, and −5.35% respectively (Table 3).

The recovery of DLM at the low, mid, and high QC levels was 113.32%, 112.22%, and 111.24% respectively. The recovery of DM-6705 at the low, mid, and high QC levels was 101.45%, 103.86%, 97.99% respectively (Table 3).

3.2.5 Injection Repeatability—The CV for each QC level was < 2% for both analytes.

3.2.6 Carryover—No signal above the threshold signal-to-noise ratio of three was detected in any of the matrix blank samples for either analyte.

3.3 Patient Hair Testing

We detected both DLM and DM-6705 in all 12 RR-TB patient samples taking DLM (Table 4). Except for one DLM concentration below the LLOQ, all concentrations were within the quantifiable range for both analytes. For DLM, sample concentrations ranged from 0.004 ng/mg to 0.264 ng/mg, with a median concentration of 0.012 ng/mg. For DM-6705, sample concentrations ranged from 0.412 ng/mg to 12.041 ng/mg, with a median concentration of 2.682 ng/mg.

We observed essentially no correlation (r= −0.05) between the concentrations of DLM and DM-6705 in participant hair samples. We also analyzed the correlation between time-on-drug and drug concentration. For DM-6705, we obtained a Pearson's correlation coefficient of 0.80 (p=0.003) that was further verified by performing Kendall's rank correlation (τ= 0.67, p=0.002). For DLM, the Pearson's and Kendall's correlations were −0.13 and −0.21 respectively (Figure 2).

4. Discussion

Delamanid will soon play an important role in newer all-oral drug-resistant TB regimens. We describe here the first assay to analyze DLM and its primary metabolite DM-6705 in small hair samples as an objective biomarker of adherence. Medication levels in hair samples can integrate behavior (adherence) and biology (pharmacokinetics), and in the context of HIV treatment and prevention, are predictive of outcomes [29]. Given the need for adherence monitoring of anti-TB drugs during the prolonged duration of treatment required for RR-TB, this hair assay should have utility for an increasingly prevalent infection.

4.1 Method Development

LC-MS/MS methods incorporating the analysis of DLM's metabolites, especially DM-6705, have been developed for serum and plasma [15,22]. The LLOQs achieved in these assays range between 1–6 ng/mL. The plasma half-life of DLM is 30–38 hours, while its

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metabolites have half-lives ranging from 121 to 322 hours [30,31]. During sample preparation, DLM is shown to be significantly converted to its metabolites under basic conditions while elevated temperature allows metabolism regardless of pH [22]. Hence, LC-MS/MS methods for plasma and serum extract DLM at low temperature under neutral conditions. Similar LLOQs are achieved for DLM and its metabolite DM-6705. Unlike DLM, however, DM-6705 does not undergo extensive metabolism during sample extraction.

We initially tried to incorporate DLM into a multi-analyte panel of eleven multidrug resistance (MDR)-TB drugs in hair as described in our previous publication [24]. While that method had an LOD for DLM of 0.005 ng/mg, two patients taking DLM in directly observed therapy showed levels in hair barely above this established LOD (0.019 and 0.020 ng/mg hair). The very small separation between the LOD and the observed DLM levels would have limited utility for the multi-analyte panel in monitoring regimens that incorporate DLM. Attempts to improve the method for DLM, however, would be quite challenging considering the LOD is already very low. Metabolites occasionally incorporate into hair to a greater extent than their parent compounds [32]. This served as impetus for incorporating the primary metabolite of DLM, DM-6705, into the multi-analyte panel. Addition of DM-6705 yielded very low, broad peaks for this analyte. Because any adjustment of the method to improve this assay also affected the assay for other drugs in the MDR-TB panel, we moved on to develop a separate method for DLM and DM-6705, described here.

In developing the new method, we adopted the same mass spectrometric parameters established for DLM and DM-6705 in the MDR-TB panel. The transitions we used have been reported in other published methods (Table 1) [15,22,23]. The previous panel provided sharp peaks for DLM so we used the same C18 reversed-phase chromatographic column, Phenomenex Synergi Polar. However, we significantly shortened the chromatographic run from 17 to 5.5 minutes and changed the elution gradient to improve DM-6705's peak shape. We achieved a slightly improved LLOQ for DLM compared to the MDR-TB panel (0.003 vs 0.010 ng/mg). More importantly, we achieved sharp peaks for DM-6705 and an LLOQ of 0.03 ng/mg hair (Figure 1). Due to the lack of stable isotope-labeled internal standards for DLM and DM-6075, we chose the antipsychotic agent OPC 14714 due to its similar molecular weight and retention time to DLM and DM-6705.

4.2 Method Validation

Validation of any LC-MS/MS-based method requires demonstration of a precise and accurate assay with high specificity, negligible matrix effect, and reproducibly high recovery. Reproducible linear dynamic ranges (700, ULOQ/LLOQ) for both analytes in our hair assay were achieved without any carryover from injection of analytes close to the ULOQ. These validation parameters were achieved after substantial changes to the original extraction protocol for the MDR-TB panel. Although that extraction protocol worked well for 11 drugs in the MDR-TB panel, it suffered from poor precision and recovery rates for DLM and DM-6705. To improve these parameters, the following modifications were made: (1) elimination of evaporation after extraction with methanol; and (2) dilution with water to 50% methanol after a single extraction.

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Evaporation after double extraction with methanol in the original method allowed for concentration of the analytes. However, we found that evaporation had a profound negative effect on the precision and recovery of DLM and DM-6705 in the new method. Because of the reported sensitivity of DLM to higher temperature in the plasma assay [15], we also tested lowering the temperature of evaporation, but neither this nor lowering the temperature of extraction improved precision and recovery of the assay. Because the metabolism of DLM during sample extraction in plasma is proposed to be albumin-mediated [33], the lack of albumin in hair may explain the stability of our assay compared to that in plasma. It was only after elimination of evaporation that precision and recovery improved.

Dilution of the extract in 50% methanol is required to facilitate reversed-phase chromatography. Elimination of evaporation did not allow concentration of the extract nor reconstitution into a more aqueous solvent. Despite the lack of concentration, the new method improved the sensitivity of the assay. Concentration also increases the amount of potential interferences in the extract. This may pose signal suppression that overrides the gain in signal from concentration. Elimination of the evaporation step increased the intensity of the peaks for both DLM and DM-6705 and lowered the noise during the chromatography phase, resulting in increased sensitivity.

4.3 Patient Hair Testing

Despite the extremely low LLOQ (0.003 ng/mg), one patient hair sample has DLM concentration <LLOQ and the median concentration observed for 12 patient hair samples was 0.012 ng/mg (Geometric mean = 0.016 ng/mg). In contrast, DM-6705 was quantified in all patient hair samples despite its higher LLOQ (0.03 ng/mg), and its median concentration was 2.682 ng/mg (Geometric mean = 2.246 ng/mg) across patient samples. All concentrations measured in patient hair samples (0.418–12.041 ng/mg hair, Table 4) fall within the linear dynamic range of the assay for DM-6705. In the plasma the typical steady-state concentration of DLM ranges between 340–410 ng/mL at an intake dose of 100 mg twice daily, while that of DM-6705 ranges between 100–220 ng/mL [30]. The reversal of the concentration ratio that we observe in hair cannot be due to a potential loss of DLM to metabolism during sample extraction as both analytes have high recovery rates. This ratio suggests better penetration of DM-6705 than DLM into hair. This reversal has also been observed for other drugs [34] and may be partially explained by higher rates of melanin binding in more basic drugs [35]. DM-6705 has a higher pKa than does DLM, so this same phenomenon may help explain the higher levels of DM-6705 in hair, although there may be various mechanisms driving incorporation into hair [34]. This observation warrants further study that is beyond the scope of developing this assay.

The lack of correlation between DLM and DM-6705 concentrations in participant hair samples is most likely because there are seven other metabolites of DLM reported in plasma that we did not measure using our method [22]. Furthermore, while we observed no correlation between DLM and time on drug, we observed a strong positive correlation between DM-6705 and time on drug, despite having corrected the measurements for the estimated amount of blank hair segments in the samples. This observation along with the fact that the lowest DM-6705 concentration observed for the participant samples is 140X

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higher than the LOD (0.003 ng/mg) and its median concentration is 894X higher clearly demonstrate that adherence to a regimen incorporating DLM is better monitored via hair levels of its metabolite DM-6705 and justifies its inclusion in our assay. Furthermore, some reports using plasma have indicated the potential role of DM-6705 for QT prolongation, an unwanted side effect of DLM [36]. With the longer timeframe readout afforded by hair levels, this potential association can further be investigated using our assay.

4.4 Limitations

Our study has several limitations. As discussed previously, we only measured one metabolite of DLM, making it difficult to predict if the other metabolites may incorporate at even higher levels than DM-6705. Additionally, our ability to interpret the hair levels we measured is limited because we lack the appropriate references for interpretation. Without careful pharmacokinetic study where hair and blood samples are collected after directly observed therapy, it is hard to correlate hair and plasma levels. One way to use DM-6705 for adherence monitoring would be to establish a threshold level that would serve as a cutoff for judging whether a patient is adherent to the drug regimen. The hair levels we have measured from 12 participants on directly observed therapy offer initial insight into expected levels of DLM and DM-6705 in hair if patients are fully compliant with their regimen, but a much larger study would be required to establish adherence cut-offs.

5. Conclusion

In conclusion, we describe the first method to analyze DLM and its metabolite in hair. This method adds to a growing number of assays we have developed to quantitatively monitor TB drugs in small hair samples [17–20]. There is a wide variety of chemical classes currently being used for drugs in RR-TB treatment, all of which cannot be compatibly incorporated into a single panel. Our current work demonstrates that a separate panel is likely to be required for specific drugs, such as DLM, for effective monitoring. Finally, as with some drugs analyzed in forensics, our strategy of targeting a drug's metabolite for analysis when its parent structure does not incorporate well into hair has proven effective for DLM monitoring. In the future, we hope to show the clinical utility of this assay to monitor DLM and its metabolite for adherence monitoring in the setting of RR-TB.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

RR-TB	rifampin-resistant tuberculosis

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DLM	delamanid
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LOD	limit of detection
TB	tuberculosis
HPLC	high performance liquid chromatography
MPA	mobile phase A
MPB	mobile phase B
QC	quality control
CV	coefficient of variation
LLOQ	lower limit of quantitation
ULOQ	upper limit of quantitation
MDR-TB	multidrug-resistant tuberculosis

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Highlights

- LC-MS/MS assay for delamanid (DLM) and its metabolite DM-6705 in hair was validated
- Delamanid was detected at very low levels in patient hair samples
- DM-6705 had levels 100X > DLM and is a better biomarker for long-term adherence

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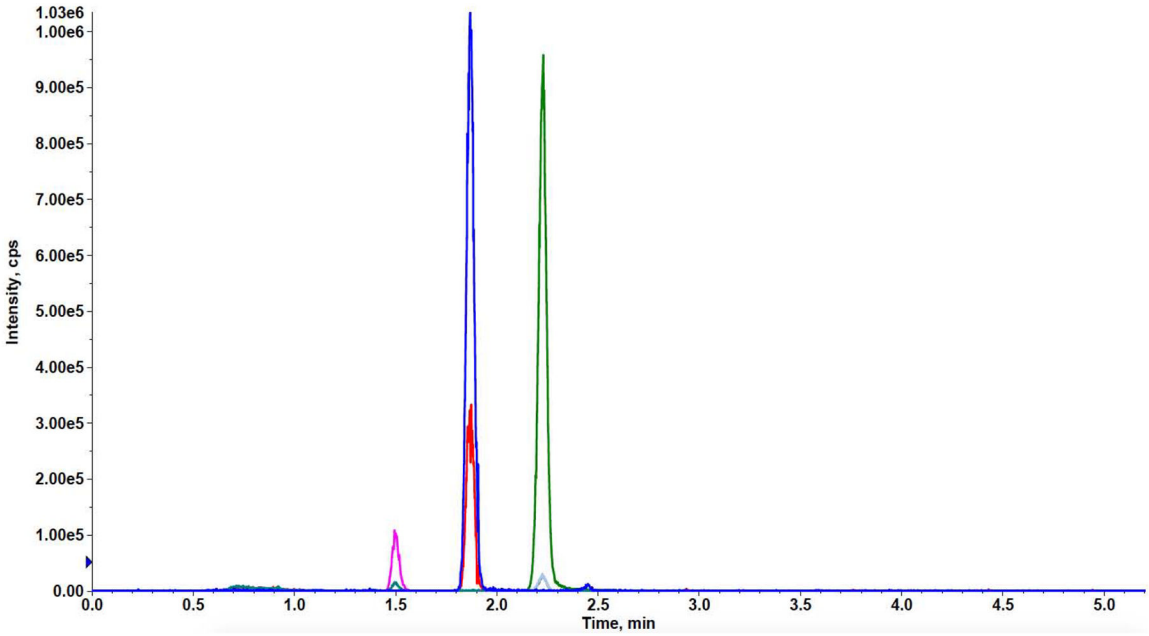

Figure 1.

Representative chromatogram of DLM, DM-6705, and OPC 14714 (internal standard). 1.5 ng mg-1 DM-6705, 0.15 ng mg-1 DLM, 16 ng mg-1 OPC-14714. DLM: delamanid.

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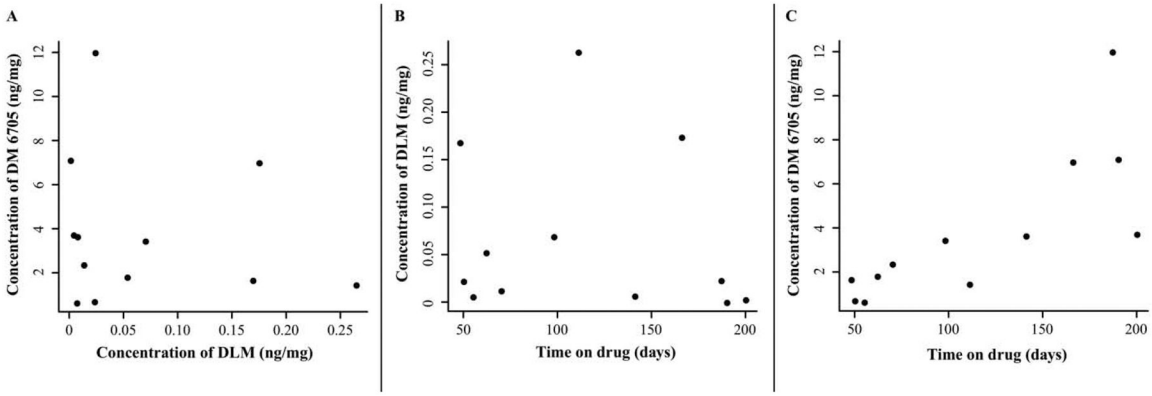

Figure 2.

(A) Correlation between hair DLM and DM-6705 levels. (B,C) Correlation between DLM and DM-6705 level respectively and study participant's time on drug. DLM: delamanid.

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Table 1.

Mass spectrometer transitions and parameters for DLM and DM-6705

ID	Q1 Mass (Da)	Q3 Mass (Da)	Time (msec)	DP (volts)	EP (volts)	CE (volts)	CXP (volts)
DM-6705-1	466.3	352.2	20	151	12	23	30
DM-6705-2	466.3	200.2	20	136	12	23	30
DLM-1	535.0	352.0	10	36	10	33	22
DLM-2	535.0	175.0	10	36	10	55	14
OPC 14714-1	459.2	296.0	2	20	10	33	20
OPC 14714-2	459.2	176.0	2	20	10	39	14

DP: declustering potential, EP: entrance potential, CE: collision energy, CXP: collision cell exit potential, DLM: delamanid

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Table 2.

Mean precision and accuracy for DLM and DM-6705 within and between the three runs.

		Within-run				Between-run			
Drug	**Parameter**	**LLOQ**	**Low**	**Mid**	**High**	**LLOQ**	**Low**	**Mid**	**High**
DM-6705	Precision (% CV)	2.34%	2.10%	1.32%	3.12%	2.53%	3.30%	2.11%	5.36%
	Accuracy (% RE)	9.45%	5.74%	11.04%	4.59%	8.44%	9.00%	12.94%	5.88%
DLM	Precision (% CV)	3.81%	4.24%	2.87%	4.06%	3.47%	8.64%	8.40%	7.61%
	Accuracy (% RE)	15.64%	8.89%	5.56%	5.30%	11.82%	8.01%	7.94%	8.92%

DLM: delamanid, RE: relative error

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Table 3.

Matrix effect and recovery for DLM and DM-6705.

Matrix Effect (%)						
Drug	**Low**	**CV**	**Mid**	**CV**	**High**	**CV**
DM-6705	2.04%	7.40%	2.18%	5.84%	−5.35%	2.95%
DLM	−0.03%	5.59%	−4.02%	6.69%	−7.86%	4.45%
Recovery (%)						
Drug	**Low**	**CV**	**Mid**	**CV**	**High**	**CV**
DM-6705	101.45%	6.51%	103.86%	5.50%	97.99%	4.19%
DLM	113.32%	11.24%	112.22%	5.16%	111.24%	4.73%

DLM: delamanid

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Table 4.

Hair levels of DLM and DM-6705 in patients taking DLM under directly observed therapy (ng/mg)

	Duration on Treatment (days)	DLM (ng/mg hair)	DM-6705 (ng/mg hair)
DLM-1	55	0.007 *	0.684 *
DLM-2	141	0.007	3.690
DLM-3	50	0.023 *	0.742 *
DLM-4	48	0.169 *	1.707 *
DLM-5	165	0.175	7.046
DLM-6	200	0.004	3.764
DLM-20	70	0.013 *	2.410 *
DLM-22	98	0.007	3.489
DLM-112	190	<LLOQ	7.158
DLM-113	187	0.024	12.041
DLM-114	11	0.264	1.492
DLM-115	62	0.053 *	1.854 *
Median	105	0.012	2.682
Geometric Mean	100	0.016	2.246

* sample concentration is corrected for shorter treatment regimen. DLM: delamanid