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Development and optimization of a gas chromatography/mass spectrometry method for the analysis of thermochemolytic degradation products of phthiocerol dimycocerosate waxes found in *Mycobacterium tuberculosis*

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RATIONALITY: The phthiocerol dimycocerosates (PDIMs) are certain stable and hydrophobic waxes found in the cell membrane of *Mycobacterium tuberculosis*, bacteria that cause an infectious disease of growing concern worldwide. Previous studies report the analysis of derivatives of the hydrolysed PDIMs from biological samples, following complex extraction and offline derivatization of PDIMs biomarkers, prior to their analysis by gas chromatography/mass spectrometry (GC/MS).

METHODS: We developed and optimized a GC/MS method based on selected ion monitoring (SIM) to detect the derivatives produced via the thermally assisted hydrolysis and methylation (THM) of the PDIMs from the cell membrane of *M. tuberculosis*. The extraction of PDIMs from culture is simple, and their thermochemolysis is carried out automatically online, thus avoiding the time-consuming derivatization steps of hydrolysis and esterification, usually performed offline.

RESULTS: For standard PDIMs in petroleum ether, our optimized method gave an excellent linearity ($R^2=0.99$) at concentrations between 0.172 and 27.5 ng/mL, a good precision (RSD = 11.42 %), and a limit of detection (LOD) of 100 pg/mL. For the PDIMs extracted from dilutions of *M. tuberculosis* culture, the method gave good linearity ($R^2=0.9685$) and an estimated LOD of 400 CFU/mL (CFU = colony forming units) in sterile distilled water.

CONCLUSIONS: A GC/MS(SIM) method is presented for the rapid and quantitative detection of *M. tuberculosis*, based on online thermochemolysis of lipodic biomarkers extracted from the bacterial culture. The method has the potential to be applied in human and veterinary clinical laboratories for the rapid diagnosis of tuberculosis in infected biological samples. © 2013 The Authors. Rapid Communications in Mass Spectrometry published by John Wiley & Sons, Ltd.
Gas chromatography combined with mass spectrometry (GC/MS) could offer a more rapid test for the diagnosis of TB by detecting lipid markers for the *M. tuberculosis* complex in biological matrices like sputum, bone, and tissue. Tuberculostearic acid (TBSA) is the most widely studied lipid marker, by the GC/MS analysis of its methyl ester (TBSAM), following the offline derivatization of the lipids extracted from culture and sputum.16–13 A major issue with analysing this biomarker is its complex extraction from biological samples, employing chemical transformations and large quantities of solvents. The alkylation of TBSA can also be performed directly on sputum via online thermochemolysis,12 but our results based on this procedure13 showed poor sensitivity and specificity, as a result of TBSAM co-elution with derivatives of matrix compounds.

We have therefore considered other lipid biomarkers that would allow a more simple extraction from culture and sputum samples, and a reduced interference from the sample matrix. In this study, we have focused on phthiocerol dimycocerosates (PDIMs), certain highly stable and extremely hydrophobic waxes that are characteristic components found only in the cell envelopes of the *M. tuberculosis* complex members. PDIMs have been reported as specific markers for tuberculosis,14–24 unlike TBSA, which can also be found in non-tuberculosis mycobacteria. The major PDIMs from the *M. tuberculosis* complex are based on C34 and C36 phthiocerol A diols, as shown in Fig. 1(a), accompanied by minor amounts of the related phthiocerol B and phthiodiolone A components (not shown here).15,16 The diol units in the phthiocerols are di-esterified with combinations of C20, C22, and C32 mycocerosic acids. Thermochrometic degradation of the PDIMs mainly produces the mycocerosic acid methyl esters C20, C22, and C32, with molecular masses of 452, 466, and 494 Da, respectively, as shown in Figs. 1(b)–1(d).

An advantage of the PDIM waxes, over other lipid entities, is their exceptionally low polarity, thus being preferentially extracted by non-polar solvents,16,20,21 which separates them from the majority of other mycobacterial and mammalian lipids. An early publication described a GC/MS selected ion monitoring (SIM) method to detect the presence of both TBSA and the mycocerosic 2,4,6,8-tetramethyloctacosanoic acid (C32) in 5-day-old cultures of sputum from patients with pulmonary tuberculosis.22 Robust chemical methods were used to degrade the extremely hydrophobic PDIM waxes to their constituent mycocerosic acids and phthiocerol diols. One such method is based on complete alkaline hydrolysis using non-aqueous potassium hydroxide/methanol/toluene, although this is time-consuming.14,15 The phthiocerol dimycocerosates can be efficiently cleaved by reduction, but again the process is complex and lengthy.16,17 Previous studies described the analysis of derivatives of the hydrolysed PDIMs from biological tissue and sputum, following extraction and offline derivatization, by using GC separation and electron capture detection (ECD) or negative ion chemical ionisation (NICI)-MS14,18–20.

In the present work, we demonstrate the development and optimization of a GC/MS method for identifying the methyl mycocerosates released by the online alkylation performed by rapidly heating the sample extracts with tetramethylammonium hydroxide (TMAH), leading to the formation of methyl ester derivatives by transesterification. The usual complex chemical manipulation of culture and sputum samples can therefore be avoided by performing the thermally assisted hydrolysis and methylation (THM) in a programmable temperature vaporizer (PTV) inlet. Our THM-GC/MS(SIM) based detection of methyl mycocerosates is intended as a diagnostic test for mycobacteria belonging to the tuberculosis family, *M. tuberculosis* and *M. bovis*, in human and veterinary clinical laboratories. In addition, it was shown that the thermochrometic methylation reaction also produces the lower volatility dimethyl ethers of the phthiocerol A moiety, which can also be profiled by GC/MS.

**EXPERIMENTAL**

**Materials and methods**

**Standards and reagents**

The standard mixture of phthiocerol dimycocerosates used in this study was extracted and purified from *M. tuberculosis* strain C, as described previously by Mallet et al.17 The standard mainly contains: (1) trimethyl- and (2) tetramethyl-branched hexacosanoic (C26 back bone) fatty acids, and (3) tetramethyl-branched octacosanoic (C28 back bone) fatty acid, whose methyl esters are shown in Figs. 1(b)–1(d), as well as the phthiocerol A diols C34 and C36 shown in Fig. 1(a). The quantitative analysis in this study will be reported relative to the total amount of the PDIMs present. Solutions of PDIMs were prepared, containing between 0.172 and 27.5 ng/mL in petroleum ether (60–80 °C, pesticide residue grade, Distol) from Fisher Scientific (Loughborough, UK) as a solvent. Tetramethylammonium hydroxide (TMAH) (methanolic 25%, Acros) from Sigma-Aldrich (Gillingham, UK) diluted to 12.5% in MeOH (pesticide residue grade, Distol) from Fisher Scientific, was used as the reagent for the online thermally assisted hydrolysis and methylation of the lipids.

![Figure 1](image-url)
Mycobacterium tuberculosis culture

The *M. tuberculosis* culture (laboratory strain C37) was grown on a 7H9 medium for 25 days, and Miles & Misra plate count[25] gave 2.8 x 10^4 colony forming units (CFU) per millilitre (mL) of neat culture. Aliquots of 1 mL of bacteria cultures were stored frozen. Prior to the extraction of the PDIMs, the culture deposits were defrosted and vortexed. Volumes of up to 200 μL of culture suspensions were then prepared, as follows: (a) 200 μL neat culture; (b) 100 μL neat culture with 100 μL sterile distilled water (SDW); and (c) 20 μL neat culture with 180 μL SDW. The culture dilutions (a)–(c) were vortexed prior to the extraction of lipids described below.

Apolar lipid extraction

The apolar lipids were extracted from the 200 μL culture suspensions (a)–(c) above by using a modified[26] method from Dobson et al.[21] 1.8 mL of MeOH and 1 mL of petroleum ether were added to each volume of 0.2 mL culture suspension, mixed on a tube rotator for 15 min, then centrifuged for 1 min to separate the liquid layers. The upper petroleum ether layer containing the apolar lipids was collected and stored at 4 °C prior to the thermochemolytic GC/MS(SIM) analysis.

Equipment

The key instrumentation in this study was an Agilent 7890A gas chromatograph equipped with a DB-5MS capillary column (15 m x 0.25 mm x 0.25 μm), coupled with a 5975C quadrupole mass spectrometer (Agilent Technologies, Queensferry, UK). The mass spectrometer was operated in electron ionization (EI) full scan mode with 70 eV electron energy, at 35 μA emission current, to provide the mass spectra for the mycocerosic acid methyl esters, and for the methylated thermochemoysates of the phthiocerol A diols from the PDIMs standard, and in selected ion monitoring (SIM) mode in the optimized analytical method targeting the mycocerosic acids methyl esters: C29, C30 and C32.

The THM reaction was performed in an Optic3 PTV inlet equipped with a PAL-CTC autosampler modified with a LINEX liner exchanger, both instruments being purchased from ATAS GL International (Eindhoven, The Netherlands).

Thermochemolysis and GC/MS analysis

Aliquots of the PDIM solutions (50 μL) in petroleum ether, and of the petroleum ether extracts from culture suspensions, were manually applied to a quartz wool plug, inside separate glass liners purchased from ATAS-GL. The solvent was evaporated to dryness for about 10 min on a hot plate at 60–70 °C, and the liners were placed in the LINEX tray. Each liner was then loaded into the PTV inlet, using the autosampler, and automated injection of 40 μL methanolic TMAH (12.5%) onto the sample was sequentially followed by the slow evaporation of the solvent prior to starting the thermochemolysis reaction. In the optimized THM method, the inlet temperature was increased from 70 °C to 380 °C, at a rate of 4 °C/s, during the derivatization of lipids. The PTV inlet temperature was held constant for 10 min, then dropped to 200 °C, to reduce thermal stress. The gas chromatograph was operated isothermally at 50 °C for 8 min, and the temperature was then increased at 30 °C/min to 350 °C, with helium as the carrier gas at a constant flow rate of 1.1 mL/min.

In the optimized GC/MS method, during the retention time window of the target compounds, between 16.4 and 17.3 min, the mass spectrometer was programmed in SIM mode, to collect the fragment ions at m/z 101, 88, and the molecular ions at m/z 452, 466, and 494. The mass spectrometer settings were adjusted to acquire the optimal average of 20 data points across a chromatographic peak. Based on the Autotune file data, the peak width at half height, Pw50, was 0.6 m/z units, as measured for the fragment ions at m/z 69 and 219 from the mass spectrometer calibration standard perfluoro-tri-butylamine (PFTBA).

RESULTS

The mass spectrum of methyl mycocerosates was generated by analyzing a 50 μL aliquot of a 137.5 ng/mL standard solution of PDIMs in petroleum ether. Hence, 6.8 ng of PDIMs in total were submitted to the thermochemolytic GC/MS method, as described in the Experimental section. Alkaline thermochemolysis causes racemization of the 2-methyl-branched mycocerosates, to give a mix of diastereoisomers due to the presence of the methyl-branched centre at carbon-4. The diastereoisomers from each mycocerosate are partially separable on the GC column, and provide very characteristic doublets,[17] as observed in the chromatogram shown in Fig. 2, generated by extracting the fragment ions of m/z 88 and 101 from the full scan mass spectra. The earlier eluting compounds are the trimethylhexacosanoic acid and the tetramethyl hexacosanoic acid methyl esters, whose individual doublets overlap to generate the typical composite C29/C30 doublet peak. The tetramethyloctacosanoic acid methyl ester (C32), whose diastereoisomers produce the second doublet peak, follows 30 s later. A much less pronounced doublet is observed for each of the molecular ions monitored at m/z 452 (C29), m/z 466 (C30), and m/z 494 (C32). The chromatographic profile for the molecular ions is amplified

Figure 2. Extracted ion chromatograms (EICs) obtained following the thermochemolysis of 6.8 ng PDIMs standard for: (a) the fragment ions at m/z 88 and 101, and the molecular ions at m/z 452, 466 and 494; (b) Insert: EICs for the molecular ions at m/z 452 (C29), 466 (C30), and 494 (C32). Note: m/z 452 and 466 refer to both peaks in the overlapping doublets for the C29/C30 methyl mycocerosates.
in Fig. 2(b), and Fig. 3 presents the corresponding fragmentation patterns obtained for the mycocerosic acid methyl esters released from 6.8 ng total PDIMs.

The extracted ion chromatograms generated for the methylated phthiocerol A components from 6.8 ng of PDIMs are illustrated in Fig. 4(a). These di-methylated derivatives of the main phthiocerol A components C34 and C36, elute at high oven temperatures of 348 °C and 350 °C, respectively, which increased the thermal stress for the DB-5MS column used, and also extended the analysis time to 21 min. The chromatographic profiles are shown for the fragment ions at m/z 201 (grey trace) and the higher mass fragment ions of m/z 339 and 367 (black traces). Their 70 eV EI molecular ions, expected at m/z 554 (methylated C34) and 582 (methylated C36), were not observed in the mass spectra.

For the development and optimization of the analytical method, we focused on the GC/MS(SIM) response obtained for the fragment ions m/z 101 and 88, each giving the two characteristic doublet peaks in C29/C30 and C32, in the retention time window between 16.4 and 17.3 min. A typical extracted ion chromatogram for these two fragment ions, for a solution of PDIMs of concentration 2.75 ng/mL of standard in petroleum ether, is shown in Fig. 5. This concentration is similar to that obtained for the PDIMs released in most of the sputum extracts in 1 mL of petroleum ether. For quantitative analysis, the m/z 101 doublet peak area was measured at both retention times, using the manual integration facility in the Agilent ChemStation software. Daily runs of the standard of PDIMs allowed for retention time alignment and correction, whenever chromatographic drift occurred. Each programmable parameter of the PTV inlet was studied to optimize the GC/MS(SIM) response for a solution of PDIMs of 2.75 ng/mL concentration in petroleum ether, hence for 137.5 pg of standard per liner.

**Transfer time**

Figure 6(a) presents the results obtained when the time taken to transfer the methylated derivatives to the capillary column was varied in the range 2–5.5 min, with the other parameters set as follows: 70 °C start and 380 °C final temperature, 12 °C/s temperature ramping rate, and a standard flow 1.1 mL/min of He carrier gas.

**Temperature ramp rate**

The influence of the temperature ramp rate was examined in the range 4–30 °C/s, by keeping all the other parameters constant: 70 °C start temperature, 360 °C final temperature, 4 min sample transfer time, and 1.1 mL/min helium flow. The results are shown in Fig. 6(b).

**Final temperature**

Following the automated injection of the TMAH solution, the PTV inlet was programmed to determine the optimum thermochemolysis temperature, with a 4 min transfer time and 1.1 mL/min He flow. The inlet temperature started at 70 °C and it was then ramped at 4 °C/s, up to final temperatures ranging between 300 and 400 °C, as presented in Fig. 6(c).

**Carrier gas flow rate**

Figure 6(d) shows the average GC/MS(SIM) response versus carrier gas flow rates of 1.1, 1.3, and 1.6 mL/min, while the other inlet parameters were held constant: 70 °C start temperature, 4 °C/s ramp rate, 380 °C final temperature, and 5.5 min transfer time.

From the results shown in Fig. 6, based on triplicate measurements, the choice of parameters for an optimized THM-GC/MS(SIM) signal was as follows: (1) 70 °C start temperature and 380 °C final thermochemolysis temperature; (2) 4 °C/s temperature ramp rate; (3) 5.5 min transfer time; and (4) 1.1 mL/min carrier gas flow rate. In the optimized method, the GC oven temperature was kept isothermal at 50 °C for 8 min, then ramped at 30 °C/min to 350 °C.

Showing the method linearity, the graphs in Fig. 7 are based on triplicate measurements of the THM-GC/MS(SIM) response for serial dilutions of the PDIMs standard solutions in petroleum ether, over a range of low concentrations of between 0.172 and 2.75 ng/mL in Fig. 7(a), and at higher concentrations in Fig. 7(b).
concentrations of between 1.375 and 27.5 ng/mL in Fig. 7(b). Both data sets show excellent linearity in each concentration range, with correlation coefficients $R^2 = 0.99$, for the peak areas in both C29/C30 and C32 doublets. Given that the average ($n = 3$) signal-to-noise (S/N) ratios for 50 μL of 172 pg/mL PDIMs solution, were 4.2:1 (C29/C30) and 17.8:1 (C32), the method LOD is estimated to be 100 pg/mL of PDIMs standard in petroleum ether, hence 5 pg per liner, for expected S/N ratios of 3:1 (C29/C30) and 5:1 (C32). For a solution containing a total of 2.75 ng/mL PDIMs, the precision shown by the RSD values was 11.86% (C29/C30) and 10.98% (C32) for a same-day repeatability study ($n = 10$), while day-to-day reproducibility ($n = 17$ measurements over 10 days) also gave good precision with RSD values of 11.02% (C29/C30) and 11.31% (C32).

The THM-GC/MS(SIM) method was applied to detect the presence of PDIMs extracted from dilutions of M. tuberculosis culture. The SIM chromatograms of the fragment ions, m/z 201 and m/z 367, in Fig. 8 were obtained via thermochemolysis of 50 μL of the petroleum ether extract from 200 μL of culture dilution in SDW, containing a total of 560 CFU. This is equivalent to 2800 CFU/mL, the lowest concentration of culture analysed in this study. When applied to the petroleum ether extracts from culture dilutions, the method presented good linearity, with correlation coefficients $R^2 = 0.9685$ (C29/C30) and 0.9679 (C32), as illustrated by the results based on duplicate...
measurements shown in the graphs of Fig. 9. The fact that the calibration graphs for culture do not pass through the origin could be due to the difficulty in preparing homogenous dilutions of M. tuberculosis, because of the clumping nature of the mycobacterial culture. It may also suggest that the petroleum ether extraction of the PDIM waxes from high concentration dilutions is not as efficient as it is for the lower dilutions of culture. The procedural blank, consisting of a petroleum ether extraction applied to 200 μL of SDW, resulted in a clean chromatogram, with no significant areas measured in the retention time window for the target peaks, m/z 101 and 88.

**DISCUSSION**

The method presented allows the rapid detection of C29, C30 and C32 methyl esters of the mycocerosic acids found in the PDIMs extracted from M. tuberculosis and submitted to online derivatization with methanolic TMAH. Starting with the extraction of PDIMs from culture, to the THM-GC/MS data interpretation, performing the complete method takes approximately 1 h per individual sample. The thermochemolytic gas chromatography/mass spectrometry of purified phthiocerol dimycocerosates waxes resulted in extracted ion profiles that are characteristic for the methyl esters of mycocerosic acids found in PDIMs. Both doublet peaks at m/z 101 and 88 are visible and easily identifiable in a retention time window while contaminant peaks are considerably fewer than in the earlier eluting region, where TBSAM, the more volatile methylated ester of 10-methyldecanoic fatty acid (TBSA), elutes.

The same thermochemolytic approach has been developed for the alkylation of TBSA directly from sputum samples[12] but, since this marker is found in most mycobacterial species, specificity for the M. tuberculosis complex appeared to require the simultaneous detection of hexacosanoic fatty acid C26FA. In our earlier work on TBSA,[13] thermochemolysis applied directly to aliquots of sputum deposits resulted in high matrix

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**Figure 5.** SIM chromatograms showing the target fragment ions m/z 101 (top trace) and 88 (bottom trace) resulting from the cleavage of the major trimethyl- (C29) and tetramethyl- (C30 and C32) methyl mycocerosates obtained by the thermochemolysis of 137.5 pg total PDIMs standard, from an aliquot of 50 μL of a 2.75 ng/mL solution.

**Figure 6.** Graph of the GC/MS(SIM) response versus the thermochemolysis parameters in the PTV inlet: (a) transfer time, (b) temperature ramping rate, (c) final temperature, and (d) carrier gas flow.
background with a massive GC/MS signal for octadecanoic fatty acid methyl ester (C18FAME) often hiding that of the closely eluting TBSAM, and even causing blockages of the capillary column. In addition, low quantities of the C26FAME were observed in TMAH blanks and in negative sputum samples, thus prompting us to explore other target analytes, specific to \( M. \) tuberculosis. The mycolic acids are also potentially powerful biomarkers for tuberculosis diagnosis but their C70 to C90 size excludes the use of routine GC/MS. However, high-performance liquid chromatography (HPLC) combined with electrospray ionization (ESI) tandem mass spectrometry (MS/MS)\(^{[23]}\) provided diagnostic \( M. \) tuberculosis mycolic acid profiles from culture and sputum samples. Shui et al.\(^{[23]}\) reported very good sensitivity and specificity, but a lengthy and complex extraction process preceded the chromatographic analysis.

In the present THM-GC/MS method, the molecular ions of C29, C30 and C32 methyl esters, at \( m/z \) 452, 466, and 494, are easily detectable in the extracted ion chromatograms obtained from several nanograms of PDIMs (Fig. 2(b)). The mass spectra shown in Figs. 3(a)–3(c) suggest that only a few cleavage reactions occur at 70 eV EI ionisation, for all three analytes. The abundant fragment ions at \( m/z \) 101 and 88 are characteristic for the fragmentation of the 2-methyl-branched fatty acid methyl esters, and result from cleavages involving the carboxymethyl moiety, as shown in Fig. 3(a). Simple fission of the C–C bond at positions 3–4 generates the fragment ion at \( m/z \) 101, whereas a McLafferty rearrangement results in the formation of the fragment ion at \( m/z \) 88.

In our search for the optimum peak to easily detect the presence of PDIMs in a sample, the carrier gas flow rate was increased to 1.4 mL/min, and the final GC temperature program was extended by 2 min isothermal at 350 °C (the highest acceptable limit for this column). Thus, the mass spectra were acquired for the much lower volatility methyl thermochemolysates of the phthiocerol A diol from the PDIMs standard. Based on the molecular structures and on the mass spectra shown in Figs. 4(b) and 4(c), we suggest that the three most abundant fragment ions in each mass spectrum result from simple fission reactions, as follows: (i) the base peaks recorded at \( m/z \) 339.4 (for methylated C34) and 367.4 (for methylated C36) may result from the cleavage of the C–C bond at positions 8–9, producing the fragment ions of theoretical masses 339.3626, and 367.3939,
respectively; (ii) the second most abundant ion (62.5–91.7%) at m/z 73.0 is likely to result from the cleavage of a C=C bond at positions 2–3, generating the fragment ion of theoretical mass 73.0 (64.33); and (iii) the third most abundant fragment ion (58.6–75%) in both mass spectra at m/z 201.2 is probably formed via the simple fission of the C=C bond at positions 7–8, resulting in the fragment ion of theoretical mass 201.1854. Although these characteristic fragment ions at m/z 201.1854. Although these characteristic fragment ions at m/z 201, 339 and 367 elute in a clean region of the chromatogram, this was not studied further in this direction, to avoid thermal stress on the column, and to reduce the likelihood of its degradation during the method development and application trial. To evaluate fully their potential as diagnostic target analytes, further studies might be performed, using a high temperature resistant column.

The method emulsion was placed on the abundant fragment ions at m/z 101 and 88 in the mass spectra of the methyl derivatives of the mycocerosic acids C29, C30 and C32, as they yield a strong GC/MS signal, and elute prior to reaching the final oven temperature. To provide an increased sensitivity and to significantly reduce instrumental noise, the quadrupole analyzer was operated in SIM mode. This approach was taken, expecting that real biological samples could produce low levels of PDIMs in a high matrix background.

As the presence of M. tuberculosis is indicated by the presence of both doublet peaks in the chromatogram, the LOD of the method for PDIMs in petroleum ether extracts from culture is determined by the S/N ratio of the least abundant signal, which we found in the earlier eluting C29/C30 doublet peak. When applying the THM-GC/MS(SIM) method to 50 μL aliquots of the petrol ether extract from 200 μL of culture dilution with the lowest concentration analyzed in this study, i.e. equivalent to 2800 CFU/mL, average (n = 2) S/N ratios of 20.5 (C29/C30) and 34.2 (C32) were obtained. From this result, we estimate that, for the culture studied, the method LOD is the equivalent of 400 CFU/mL in sterile distilled water, for expected S/N ratios of 3:1 (C29/C30) and 5:1 (C32).

Our thermochemolysis-based GC/MS(SIM) method has also been applied to analyze the petroleum ether extract from a batch of 395 sputum samples of TB suspect patients, without prior knowledge of the clinical and culture classification of sputum samples in positives and negatives. Details of the analytical issues encountered in biological samples with trace level of analytes in a complex matrix background, as is the case of sputum sample extracts, were discussed in an earlier publication that also emphasized the clinical aspects of our study. Relative to the culture, the final results showed 64.9% sensitivity and 76.2% specificity.

CONCLUSIONS

A gas chromatography/mass spectrometry method is presented that allows the rapid and quantitative detection of M. tuberculosis in culture samples and sputum. The method relies on the mass spectral identification of the derivatives produced from the thermally assisted hydrolysis and methylation of the PDIMs extracted from the lipidic cell membrane, thus avoiding the time-consuming offline derivatization steps involving hydrolysis and esterification. Compared with other reported mycobacteria identifiers, these particular mycocerosate profiles are specific to members of the M. tuberculosis complex. The method is rapid, taking less than 1 h from lipid extraction to the THM-GC/MS result, and it shows excellent linearity, repeatability and day-to-day reproducibility for solutions of PDIM standards in petroleum ether. Very good linearity was also obtained by applying the method to the petroleum ether extracts from 200 μL dilutions of M. tuberculosis culture in sterile distilled water. The recording of GC/MS profiles for the methyl derivatives of the phthiocerol A moiety provides the potential for further study to obtain additional specificity in the diagnosis of tuberculosis, by using PDIM markers.

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