Development of a quantitative chemical ionization gas chromatography–mass spectrometry method to detect tuberculostearic acid in body fluids

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Abstract

We developed a mass spectral method to verify the detection of free tuberculostearic acid (TSA) by frequency-pulsed electron-capture gas chromatography (FPEC–GC) in cerebrospinal fluid (CSF), serum, pericardial fluid, ascites fluid and pleural fluid of patients infected with *Mycobacterium tuberculosis*. To obtain satisfactory sensitivity and specificity for comparison of the test using mass spectrometry (MS) in the single ion monitor (SIM) mode to the FPEC–GC test, we developed a specific, sensitive, quantitative chemical ionization mass spectrometry capillary gas chromatography (QCIGC–MS) test. The procedure maximized the molecular ion (i.e., made it the base peak) for increased specificity and sensitivity, and instrument parameters for increased sensitivity. The procedure uses a computerized approach, requiring an internal standard (nonadecanoic acid) for precise measurement of the retention time and quantitation of the molecular ion of TSA. Data from this study suggest that QCIGC–MS analysis could be a valuable tool to confirm FPEC–GC identification of TSA in CSF, serum, and in pleural, ascites, and pericardial fluids. Published by Elsevier Science B.V.

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1. Introduction

Laboratory confirmation of disease caused by *Mycobacterium tuberculosis* is problematic. The organism is slow-growing and, often, as in cases of tuberculous meningitis, in which the number of tubercle bacilli in spinal fluid is very low, it is extremely difficult to recover. It has been estimated that less than half of the specimens with clinical evidence of tuberculous meningitis have a positive culture [1]. Since few clinical cases of tuberculous meningitis are confirmed by culture, an accurate rapid diagnostic method is needed.

We have previously shown that frequency-pulsed electron-capture gas chromatography (FPEC–GC) is a sensitive, reliable technique for the detection of tuberculostearic acid (10-methyloctadecanoic acid, TSA) [2,3]. Femtomole quantities of TSA have been

detected in the cerebrospinal fluid (CSF) of patients with tuberculous meningitis, but in patients with unclear diagnoses, a second chemical technique to verify the presence of TSA is desirable.

In earlier studies, Elias et al. [4] and French et al. [5] extensively used electron impact mass spectrometry gas chromatography (EIGC–MS) in the single ion monitor (SIM) mode to monitor the fragments at m/z 167 and 312 of the methylester of TSA made from saponified CSF. Using the method of French et al. [5] with TSA standards, samples positive for *M. tuberculosis*, and healthy controls, our laboratory found problems in both the specificity and sensitivity with this technique. Firstly, using maximum sample injection, the technique barely detected the methyl ester of TSA above background noise, and an accurate reading for TSA required precise measurement of the retention time, which was difficult to obtain without an internal standard that eluted close to the retention time of TSA. Secondly, the molecular ion that was the major ion relating to specificity was near background noise, and the ratio of the m/z 167 fragment to the molecular ion at m/z 312 was very difficult to reproduce.

Since chemical ionization gas chromatography mass spectrometry (CIGC–MS) gave a strong molecular ion (base peak) with analysis of the TSA methyl ester, we decided to use CIGC–MS and add nonadecanoic acid (C19) as an internal standard. This would permit more reproducible detection of the retention time for TSA, based on retention time calculations using the internal standard. Additionally, it provided a reference for area quantitation of the molecular ion. Furthermore, we investigated the possibility of improving the sensitivity by increasing TSA esterification and maximizing instrument parameters.

2. Experimental

2.1. Samples

Cases of tuberculous meningitis and pulmonary and extrapulmonary tuberculosis were identified by clinical evaluation of the patient's symptoms and by culture for *M. tuberculosis* using standard techniques

[6]. When no culture was obtained, clinical cases were evaluated by a panel of tuberculosis experts using the accepted criteria [7].

Samples of CSF were collected under sterile conditions, placed in a red-top tube, without the addition of chemicals or centrifugation, frozen, and shipped to CDC (National Centers for Disease Control) for further evaluation. To obtain serum samples, 10 ml of blood were drawn under sterile conditions from tuberculosis patients and healthy controls and placed in a red-top tube. After clotting at room temperature, the serum was separated by centrifugation for 5 min at less than 500 g. The serum was then frozen without the addition of chemicals and shipped to the CDC on dry ice. Other fluids (pericardial, ascites and pleural) were also drawn under sterile conditions, placed in a red-top tube without centrifugation or the addition of chemicals, frozen, and shipped on dry ice to CDC. A brief clinical history of the patient was included.

2.2. Preparation of trichloroethyl (TCE) esters

A 2-ml volume of body fluid was extracted under acidic conditions, near pH 2 (Alkacid test ribbon, Fisher Scientific, Atlanta, GA, USA) with 20 ml of nanograde chloroform. The extract containing the carboxylic acids was then concentrated to about 20 μl, and TCE esters were prepared using heptafluorobutyric anhydride as a catalyst, as previously described by Brooks et al. [2]. The derivatized sample was resuspended in 200 μl of xylene and placed in a 200-μl autosampler glass-lined crimp-cap vial (National Scientific, Norcross, GA, USA) for autosampler injection of 2 μl into the gas chromatograph.

2.3. Preparation of methyl esters

Methyl esters were prepared as described by French et al. [5], which involved saponification of the body fluid and methylation with borontrifluoride methanol. Alternatively, methyl esters were prepared as follows: A 2-ml volume of body fluid was placed in a 50 ml Teflon-lined screw cap round-bottomed Pyrex centrifuge tube (Corning, Corning, NY, USA), acidified to near pH 2 with 0.1 ml of 50% H_2SO_4 (v/v, acid and distilled water), and then extracted with 20 ml of nanograde chloroform (Mallinkrodt,

Stone Mountain, GA, USA) on a Burrell wrist-action shaker (Burrell, Pittsburgh, PA, USA) at a setting of ten for 5 min. Next, the chloroform layer was transferred using a 10-ml glass pipette (leaving behind the fat globules) to a 150×20 mm Pyrex test tube and evaporated just to dryness with clean dry air that was filtered through Drierite (W.A. Hammond Drierite, Xenia, OH, USA) in a Meyer Analytical Evaporator (Northborough, MA, USA). The evaporator was filled with sand and maintained at a temperature of 85°C. Next, 1.5 ml of 3 M methanolic HCl were added, and the tube was sealed with a Teflon-lined screw cap, briefly hand shaken to mix, and heated in the 85°C sand bath for 15 min. The tube containing the sample was then cooled with tap water, and 4 ml of hexane were added. The sample was then shaken on the wrist-action shaker for 5 min, and the hexane (top layer, about 3.9 ml) was carefully removed using a 5-ml glass pipette and placed in a 75×10 mm disposable Pyrex culture tube (Corning) (Note: During such procedures, if any of the bottom aqueous phase is transferred, it should be removed before evaporation). The sample was then evaporated with clean dry-filtered air in the 85°C sand bath to just dryness in the test tube. Finally, 30 μl of xylene for EIGC–MS or 75 μl of xylene for QCIGC–MS were added; the sample was then hand shaken to mix and transferred to a 200-μl glass-lined crimp-capped autosampler vial for autosampler injection of 4 μl.

2.4. Determination of the optimal methylating procedure

Methyl esters of lauric acid (Chem Service, Media, PA, USA; 40 pg/4 μl injection) were prepared by the two dry methods (i.e. samples were extracted into organic solvents prior to derivatization) described above and by the addition of 1.5 ml of 3 M methanolic HCl to a 2-ml aqueous sample containing the same concentration of lauric acid, to test the effect of water on esterification. Ten different sample preparations were made for each of the two dry methods tested, and five preparations were made for the wet method. Peak areas were obtained by total ion monitor and autointegration (i.e., integration performed by the computer using mass spectrometry software) of each sample peak by analysis of the sample using the EIGC–MS mode. The area counts were then compared to area counts obtained from both EIGC–MS analyses of methyllaurate standard (Chem Service) of the same concentration. The percentage methylation was determined by this comparison as follows: Area counts from the total ion current sample peak/area counts from the total ion current sample peak of methyllaurate standard×100. An equal cell volume of buffered saline washed cells from cultures of *M. tuberculosis* (about 200 000 cells by the McFarland standard) were saponified and tested by both mass spectrometry (MS) methods to obtain an estimate of the number of cells required for detection.

2.5. Quantitative chemical ionization gas chromatography mass spectrometry (QCIGC–MS)

A computerized quantitative internal standard method for the determination of TSA was prepared using specific guidelines, as described in the Hewlett-Packard G103A MS Chemstation workbook (DOS series). The internal standard (I.S.) used was a 200-fmol/4 μl injection of methylnonadecanate (C19). Chemically, the methyl esters of C19 and TSA were similar and gave a linear response when area count was plotted against concentration. The C19 methyl ester was distinguished by a retention time difference of more than 1 min from the methyl ester of TSA (10-methyloctadecenoic acid). The computer used the response to a known amount of the C19 to correct automatically for both retention time change and area count variation of fragmentation response when they occurred and to determine the concentration of TSA.

2.6. Operating conditions for GC–MS and FPEC–GC

A Hewlett-Packard GCMS-Chem Station (5890-5971A) equipped with an autosampler (HP-7637A), which was controlled by the Chem Station computer (Vectra QS/165), was used. The mass spectrometer possessed both electron impact (EI) and chemical ionization (CI) capabilities. The 5890 gas chromatograph was also equipped with a 15-mCi ^{63}Ni electron capture detector and strip chart recorder (Perkin Elmer), which was operated at 1 mV and at a chart

speed of 10 cm/min. An IBM 9000 computer received FPEC–GC data from the gas chromatograph. The capillary column for the MS was a fused-silica cross-linked methyl silicone column that was 0.52 μm thick (OV-1) and 25 m×0.2 mm I.D. (Hewlett-Packard, Atlanta, GA, USA). Helium was used as the carrier gas at a flow-rate of 0.4 ml/min. Methane was used for chemical ionization of the methyl esters of TSA and the I.S. The column for FPEC–GC analysis was a large-bore, fused-silica, crossed-linked OV-1 5 μm thick column 25 m length×0.5 mm I.D. (Southeastern Lab Apparatus, Augusta, SC, USA). Helium was used as the carrier gas at a flow-rate of 5 ml/min. The make-up gas for the FPEC–GC detector was 95% argon and 5% methane. The instrument was also equipped with the following: (a) Splitless injectors for EIGC–MS, QCIGC–MS and FPEC–GC analysis, which were opened to vent the solvent 2 min after sample injection; (b) flow controllers and (c) deactivated glass injector liners (HP 5181-3316). The temperature program for both the mass spectrometer and FPEC–GC was 90°C for 4 min, then a linear increase at 4°C/min to 285°C, which was held isothermally for 10 min for MS or for 31.25 min for FPEC–GC. The temperature of both injectors was 250°C, and the temperature of the mass detector was

190°C. The electron capture detector temperature was 300°C. QCIGC–MS conditions for quantitative analysis of TSA were as follows: SIM parameters were used to monitor the molecular ion (which was also the base peak) and a qualifier ion. For maximum sensitivity, extracted ions were monitored at m/z 313.1 [M+1.1] and m/z 297 using low resolution, a dwell per ion of 200 ms and an electron multiplier voltage of about 1600 (relative, after standardization plus 200). The reference peak was C19, and it eluted at 41.67 min±20 s. The methyl ester of TSA (the target peak) eluted at 40.33 min±20 s. The C19 ion was computer monitored from 41.37 to 41.97 min, and TSA was likewise monitored from 40.03 to 40.63 min to detect and correct retention time drifts when and if they occurred. The computer generated a report detailing retention times and quantitative results based on the I.S.

3. Results

A preliminary study was conducted using the method of French et al. [5], hereafter referred to as ''method 1'', SIM-EIGC–MS and FPEC–GC. It became evident early in the study using body fluids (Table 1) and from experiments using 15–20 fmol of

Table 1
Comparison of FPEC–GC and method 1 for the analysis of TSA

CDC number	Sample type	FPEC–GC for TSA	Method 1 SIM-EIGC–MS	Clinical data
CA3795	CSF	+	+	tuberculous men.
CA3754	CSF	+	−	tuberculous men.
CA3756	Serum	+	?	tuberculosis
CA3709	Serum	+	?	tuberculosis
CA3709	CSF	+	−	tuberculous men.
CA3789	CSF	+	−	tuberculous men.
CA3530	Serum	+	?	tuberculosis
CA3708	Serum	+	?	tuberculosis
CA3783	CSF	+	−	tuberculous men.
CA3789	CSF	+	±	tuberculous men.

FPEC–GC=frequency-pulsed electron-capture gas chromatography.
SIM EIGCMS=single ion monitoring electron impact gas chromatography mass spectrometry.
men.=meningitis.
TSA=tuberculostearic acid.
CSF=cerebrospinal fluid.
+=Positive for TSA (15–20 fmol).
±=Weakly positive (12–14 fmol).
−=Negative for TSA.
?=Result cannot be read because of background caused by saponification.

TSA extracted from water that went undetected by method 1 that the method was insensitive. Background also was a significant problem when saponified serum was involved. It was also determined from saponification of buffered-saline-washed cells of *M. tuberculosis* that more than 200 000 organisms were necessary to detect TSA by EIGC–MS or FPEC–GC.

In order to detect 15–20 fmol per 2 ml of body fluid (the amount found most often in CSF samples from tuberculous meningitis patients and submitted to our laboratory [3]) of methyl TSA standard, we first optimized the following parameters to maximize sensitivity by analysis of TSA standards: Dwell, voltage, splitless injector and temperature programming. In addition, we used computerized background correction in both the EIGC–MS and CIGC–MS modes of operation. Furthermore, a much smaller resuspension volume was used than was used for FPEC–GC analysis (reduced from 200 to 30 μl for EIGC–MS) and the maximum volume of sample injection was used. However, even with reduced final sample volume, maximum volume injection, and operation of the instrument at optimum parameters for sensitivity, 2.8 fmol of TSA methyl ester were required per injection for detection of TSA methyl ester above background noise, as opposed to 0.2 fmol (per 2 μl injection from a 200-μl final volume of solvent) for the detection of TSA TCE ester by FPEC–GC. At these low levels of analysis for TSA methyl esters, problems were encountered other than sensitivity with EIGC–MS monitoring in the SIM mode for the ions at m/z 167 and 312. Firstly, the molecular ion (which accounted for most of the specificity) at m/z 312 was weak, and it was difficult to obtain a completely negative reading for these two ions because readings had to be taken at a sensitivity that was very close to baseline noise. The taking of background spectra just prior to elution of TSA (by use of the computer mouse or by a computer automated process) and computer extraction of the background spectra from the TSA spectra gave improved sensitivity readings by a reduction of the background noise, as determined by performing spectral readings with and without background correction. It was possible to change the peak area and peak ratio of the two monitored ions by short movements of the ion marker to the left or right of a peak that eluted where TSA was previously de-

termined to elute. Secondly, to obtain a reliable m/z reading, a correct retention time value was necessary, and it was empirically determined for TSA by the addition of an I.S. reference. Third, an I.S. was also necessary to stabilize small variations in area counts that occur in both EIGC–MS and CIGC–MS analysis modes (Hewlett-Packard G103A MS Chemstation workbook, DOS series). Area count variation, when it occurred, made it difficult to establish a reproducible background reading among controls at this low level of threshold values. Finally, as previously described [3], these problems were made worse by a reduction of TSA and increased background readings when the body fluid was saponified using method 1.

We compared ten analyses of each of the two dry methods (methods 1 and 2) for percentage yield of methyl ester. We chose lauric acid because we had previously obtained high purity methyllaurate and lauric acid from the same commercial source. For method comparison, we used EIGC–MS area counts obtained from analysis of methyllaurate prepared commercially and by method 1 using fresh boron-trifluoride methanol to the method of extraction, drying, and heating at 85°C for 15 min with 3 *M* methanolic HCl (hereafter referred to as ''method 2''). When we compared the percentage yield obtained by analysis of methyllaurate prepared by both methods with commercially purchased methyllaurate, we obtained percentage yields of methyllaurate with a mean of 40.10% and a standard deviation of 1.286 from method 1 and a mean of 63.55% and a standard deviation of 1.509 for method 2. Additionally, we determined that retrieval of methyllaurate was increased when the methyl ester was extracted with only one solvent (4 ml of hexane) compared with the two solvents used in method 1. Comparable tests with methylation of lauric acid in 2 ml of water ($n=5$) using 1.5 ml of 3 *M* methanolic HCl, as would be encountered in the direct methylation of body fluids, gave a mean of 10.1% and a standard deviation of 1.025. The maximum yield was obtained by adding methanolic HCl to the extracted dry sample (method 2).

We also found from injection of the same TSA standard ($n=5$) that starting with a low temperature (90°C), programming at a moderate rate (4°C/min), and using xylene as a final solvent produced TSA methyl ester peaks with a percent full scale height that had a mean of 50.00 and a standard deviation of

1.581. Using the same injection ($n=5$) with a starting temperature of 200°C produced peak heights of TSA methyl ester with a percent full scale that had a mean of 15.4 and a standard deviation of 1.140.

Fig. 1 shows the spectrum obtain from positive CI analysis of the methyl ester of TSA using methane as the ionizing gas in the scan mode. Note that the fragment at m/z 313.1 is both the molecular ion and the base peak in the CI mode. The increased sensitivity of the molecular ion detection was important for obtaining improved detection and specificity.
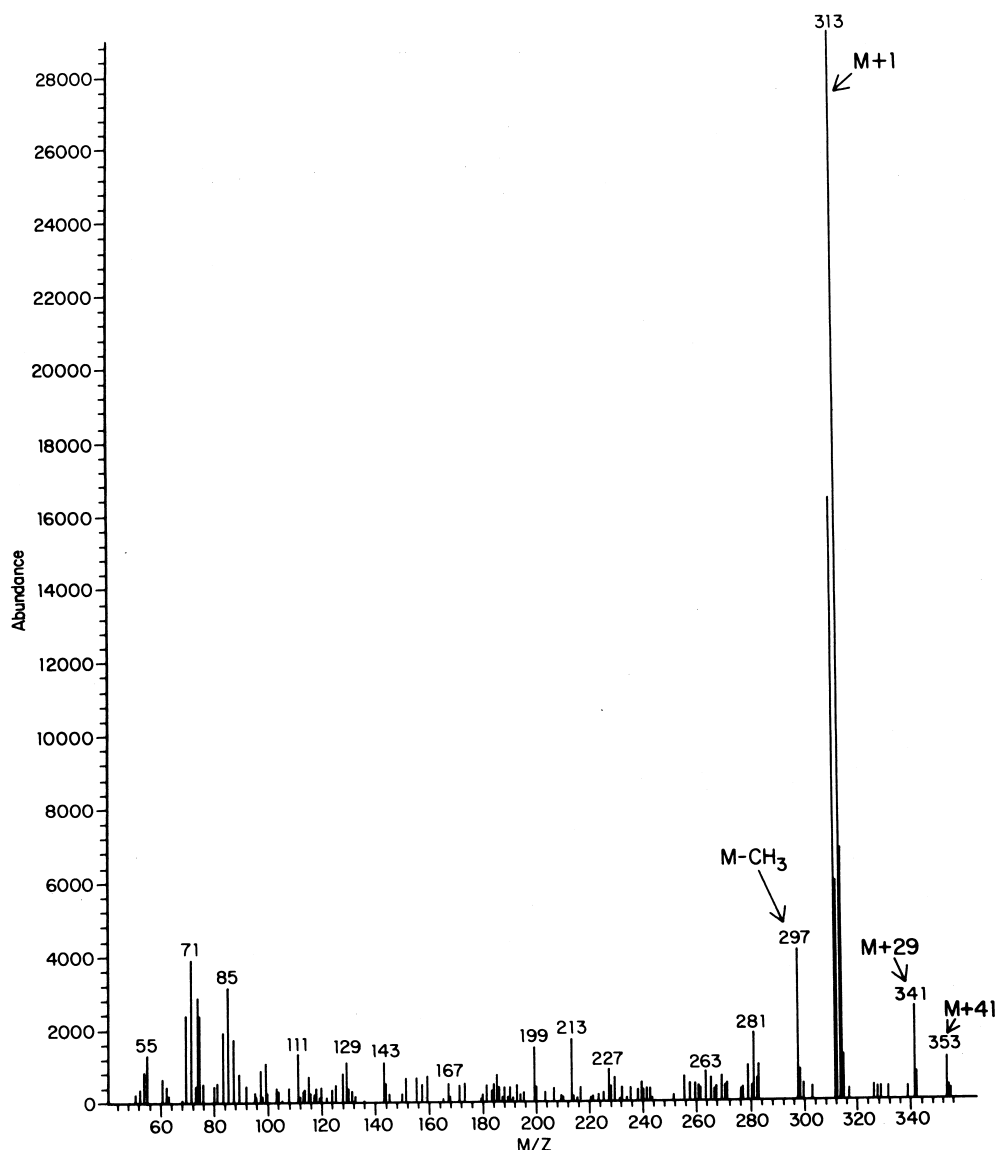
Attempts to use both CI and EI mass spectrometry

Fig. 1. Chemical ionization (CI) spectra of methyltuberculostearate. The ionizing gas was methane. The column contained crosslinked methyl silicone [0.52 μm thick (OV-1), 25 m×0.2 mm I.D.). The instrument was programmed at 90°C for 4 min, then for a linear increase of 4°C/min to 285°C, and it was held isothermal at 285°C for 10 min. The molecular ion [M+1] and the base peak are the same at m/z 313.

at maximum sensitivity to detect TCE esters of TSA at the detectable level of 15–20 fmol per 2 ml of body fluid (as was done with FPEC–GC analysis) failed to give the same sensitivity for TSA as for the methyl ester. There was a noticeable increase in the specificity of the TCE ester of TSA when monitored at m/z 429 [M+1], m/z 393 [M−35] and m/z 359 [M−69]. This was due to the presence of the chlorine isotopes, which leave a characteristic chlorine fingerprint [8] that is not obtained by monitoring the methyl ester of TSA with EIGC–MS for ions at m/z 167 and 312. Another reason for the increased
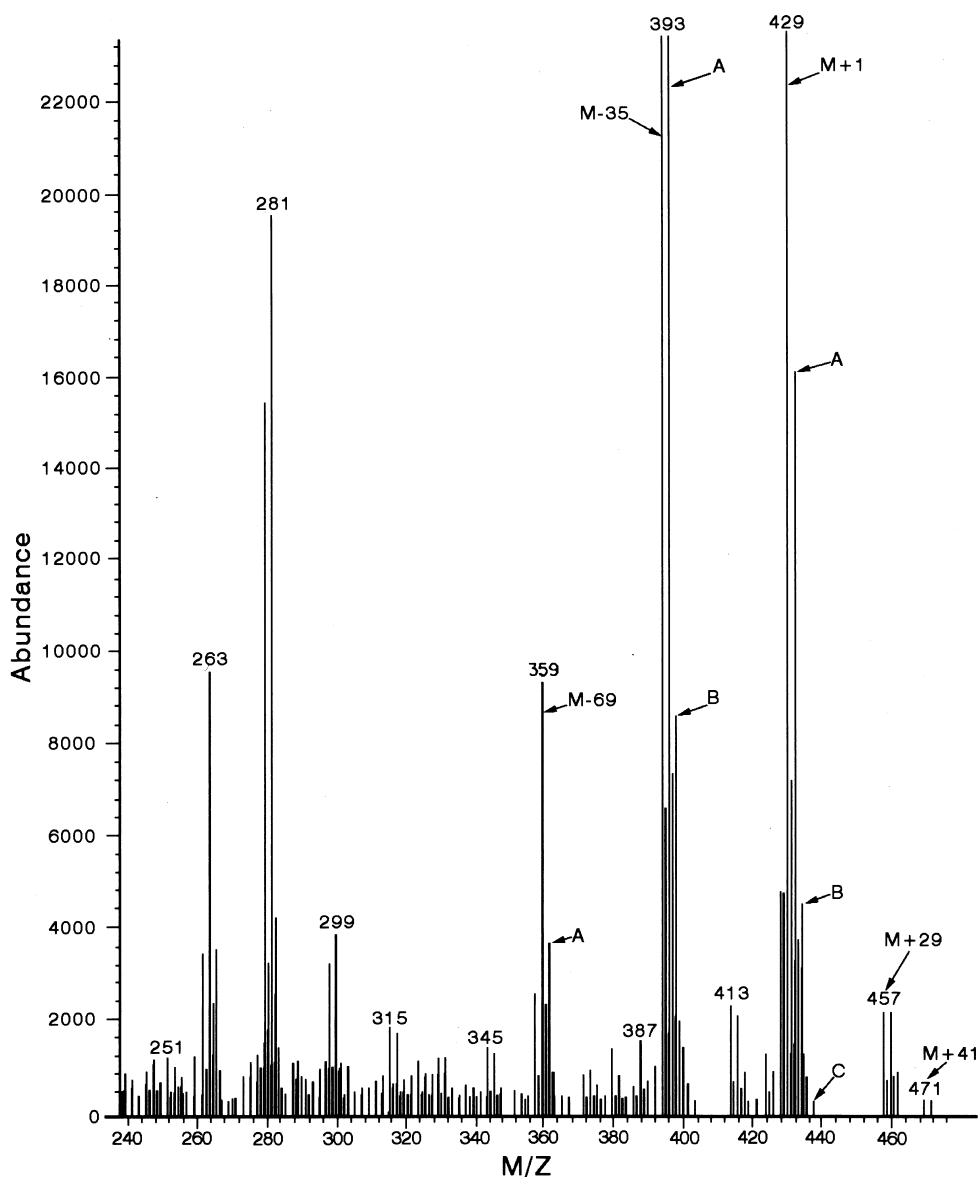

Fig. 2. Chemical ionization spectra of trichloroethyltuberculostearate. The gas chromatography and mass spectrometry conditions are the same as those in Fig. 1. The molecular ion [M+1] is shown at m/z 429. The three chlorine atoms with isotopes A, B and C are present. A second chlorine-containing fragment with two chlorines and isotopes A and B is present at m/z 393, and a third chlorine-containing fragment with one chlorine and one isotope A is present at m/z 359.

specificity was the ability to distinguish the chlorine isotopes from background noise. Sensitivity, however, was about 25% less than that obtained using the methyl ester.

Methylation of TSA and monitoring of the molecular ion with CIGC–MS at m/z 313.1 [M+1.1] increased the sensitivity because of the stronger molecular ion; however, problems with specificity and quantitation were still evident in retention time determination and area count. Use of an I.S. permitted the computer to automatically correct for the small retention time shifts and quantitative error that sometimes occurred in an analysis, due to area count fluxional characteristics. The use of QCIGC–MS, inclusion of an I.S., a target ion at m/z 313.1, a qualifier ion at m/z 297 [M−15] and computer compensations, which were based on characteristics of the I.S., were important additions that improved the specificity and sensitivity for the detection of TSA.

Fig. 2 shows the spectrum obtained by CIGC–MS analysis for the trichloroethyl esters of TSA in the scan mode. Noteworthy are the chlorine fragments of the trichloroethyl ester of TSA at m/z 359 [M−69] containing one chlorine atom and isotope A, m/z 393 [M−35] containing two chlorine atoms and isotopes A and B, and m/z 429 [M+1] containing three chlorine atoms and isotopes A, B and C. Fragment characteristics have been described previously [8].

Fig. 3A shows the total ion monitor for TSA at m/z 313.1 (top scan line) and 297 (bottom scan line); Fig. 3B shows the QCIGC–MS SIM mode spectra obtained by analysis of methyl esters of TSA in culture-positive cases. The I.S. (not shown), which eluted later than TSA by about 1 min, is higher in intensity and has identical CI SIM spectra to those of TSA.

Table 2 presents a comparison of samples analyzed by FPEC–GC and the optimum MS method, as determined experimentally in this study. The purpose of the comparison was to determine the possibility of using MS to confirm identification of the low levels of TSA detected by FPEC–GC in CSF, serum, and ascites, pleural and pericardial fluids. In each case, a 4-ml sample was split and 2 ml from each patient were analyzed by FPEC–GC and QCIGC–MS. Results from the five specimens that were culture-positive agreed 100%, and there was a 91% agree-
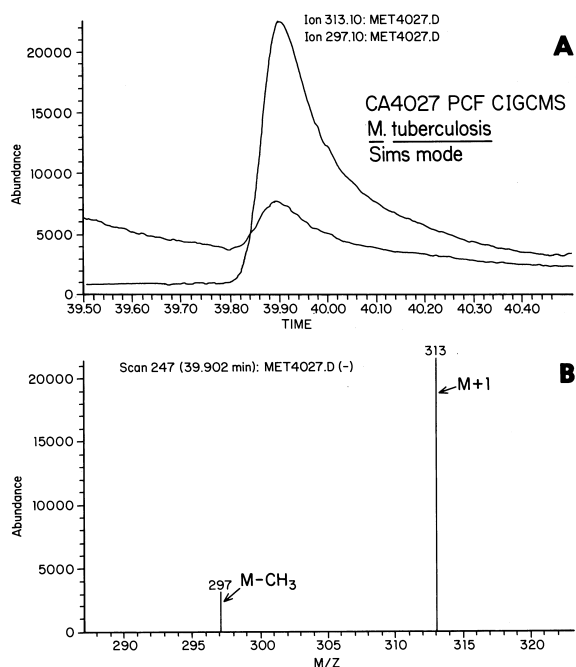

Fig. 3. Quantitative chemical ionization gas chromatography (QCIGC–MS) total ion monitoring chromatogram (A) and spectra (B) of methyl esters made from an acidic chloroform extraction of 2 ml of pericardial fluid from a culture-positive case involving *M. tuberculosis*. Single ion monitoring was used to detect ions at m/z 313.1 and 297.

ment between QCIGC–MS and FPEC–GC readings on the remaining 21 specimens.

4. Discussion

Other workers [9–12] have presented conflicting results concerning the sensitivity and specificity of EIGC–MS SIM mode analysis for TSA. We have found ([3] and unpublished data) that other factors can affect the reliability of MS or FPEC–GC tests in detecting TSA. These include therapy (often within 24 h), centrifugation of the sample, saponification of the sample, contamination of the sample followed by growth of the organism, sample amount and, in some cases, vaccination with BCG vaccine (unpublished data) can produce positive results for TSA.

The major purpose of this study was to develop a MS method to confirm identification made by FPEC–GC analysis for free TSA at the low levels

Table 2
Comparison between FPEC–GC and QCIGC–MS for analysis of TSA[a]

CDC number	Sample type	FPEC–GC for TSA	QCI–GC–MS for TSA	Culture for *M. tuberculosis*	Clinical data and comments
CA4588	CSF	+	+	−	Suspect TB men.
CA4589 (a)	CSF	+	+	−	Suspect TB men.
CA4589 (b)	Serum	+	+	ND	Same as (a)
CA4590	CSF	+	+	−	Suspect TB men.
CA4587	CSF	−	−	−	Undetermined
CA4581	Serum	+	+	−	Suspect TB
CA4524	Ascites	+	+	+	*M. tuberculosis*
CA4282	Pleural	+	+	+	*M. tuberculosis*
CA4027	Pericardia	+	+	+	*M. tuberculosis*
CA4038	CSF	+	+	+	Tuberculous men.
CA4065	CSF	+	+	+	Tuberculous men.
CA4048	CSF	+	+	−	Suspect TB men.
CA4052	Serum	+	+	−	Suspect TB
CA4409	CSF	+	−	−	Suspect TB men.
CA4545	CSF	−	−	−	Undetermined
CA4398	CSF	−	−	−	Undetermined
CA4458	Serum	+	+	−	Suspect TB
CA4323	CSF	+	+	−	Suspect TB men.
CA4441	CSF	−	−	−	Undetermined
CA4242	CSF	−	−	−	Undetermined
CA4468	CSF	−	±	−	High background
CA4228	Pleural	+	+	+	*M. tuberculosis*

FPEC–GC=frequency-pulsed electron-capture gas–liquid chromatography.
QCIGC–MS=quantitative chemical ionization gas chromatography mass spectrometry.
TB=tuberculous or tuberculosis.
M.=*Mycobacterium*.
ND=Not done.
+=Positive.
−=Negative.
[a] The level of TSA in each positive sample was between 15 and 20 fmol (10^{-15} mol)/2 ml of body fluid.

most often found in cases of tuberculous meningitis in our laboratory (15–20 fmol per 2 ml of body fluid [3] and unpublished data). The MS method chosen for study seemed to have potential as a rapid test to detect TSA, and the MS instrumentation was similar to that available in our laboratory [5]. From the beginning of the study using both TSA standards and culture-positive cases, two things were apparent; there was a lack of sensitivity for the detection of TSA in comparison to that obtained using FPEC–GC analysis, and a lack of specificity of the SIM mode of analysis for low concentrations of the methyl ester fragment of TSA at m/z 167 and 312. Since the number of tubercle bacilli in lumbar CSF in an active case of tuberculous meningitis is very low (probably less than 1000, as determined by antigen concentration and microscopic examination [13] and per-

sonal communication with Robert F. Good, CDC), there appeared to be a build-up of free TSA in the CSF, otherwise, this small number of organisms would not produce detectable amounts of TSA by saponification; therefore, saponification of the body fluid was unnecessary. The sensitivity of the MS test (method 1) was significantly improved by optimizing the parameters described above, but specificity of the test at the low femtomole level of analysis was problematic. The problem mainly consisted of a frequently encountered 167 ion (often unrelated to TSA), and the near baseline detection of the molecular ion at m/z 312, which accounted for most, if not all, of the specificity. The MS test was significantly improved by using the CI mode of analysis that produced a strong molecular ion that was also the base peak. Another significant improvement was

made by the introduction of an I.S. (C19) that eluted within 1 min of TSA and permitted more accurate computerized measurement of the retention time for elution of TSA in each analysis. A correct retention time reading was a very important factor for identifying TSA, both in EI and CI modes of operation at low femtomole levels of detection.

When a physician encounters a patient with lymphocytic meningitis and clinical characteristics of tuberculous meningitis, he faces a dilemma in choosing the type of therapy to use [14]. In one coded study [2], FPEC–GLC was found to have a specificity of 92% and a sensitivity of 95% for the detection of tuberculous meningitis. However, in smear-negative, culture-negative cases in which opinions concerning diagnosis differ, verification of TSA by a second type of analysis may assist physicians in making a final decision.

The results found in this study indicate that QCIGC–MS could be used to verify FPEC–GC detection of TSA. One possible obstacle to verification of FPEC–GC results by QCIGC–MS, however, is the requirement for 4 ml of CSF to perform both the FPEC–GC and QCIGC–MS methods. Another approach (which we did not pursue because our instrumentation did not possess the capabilities) that should be investigated is the use of negative chemical ionization MS, which might detect the chlorinated molecular ion of the TCE ester at the same level of sensitivity as CI analysis of the methyl ester. If this were the case, the same TCE ester used for analysis by FPEC–GC could also be used for MS analysis; thus, only 2 ml of CSF would be necessary for the determination of TSA by both FPEC–GC and MS. In addition, the specificity of the MS analysis could be improved because of the presence of chlorine isotopes.

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