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Comparison of ion trap and triple quadrupole gas chromatography–tandem mass spectrometry in the quantitative and qualitative trace analysis of muramic acid in complex matrices

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Abstract

During the past few years we helped introduce the use of gas chromatography-tandem mass spectrometry (GS-MS/MS) for trace detection of chemical markers for bacteria and their constituents in complex clinical and environmental matrices. A particular marker of interest is muramic acid found in bacteria but not elsewhere in nature. Absolute identification is achieved using the product ion spectrum and levels of chemical markers for bacteria determined by multiple reaction monitoring. Work directly comparing, the utility of ion trap and triple quadrupole GC-MS/MS instrumentation, in the quantitative and qualitative analysis of muramic acid, is presented here. (Int J Mass Spectrom 190/191 (1999) 321–329) © 1999 Elsevier Science B.V.

Keywords: Muramic acid; Tandem mass spectrometry; Trace analysis

1. Introduction

Certain compounds are found in particular species or genera of bacteria and serve as chemical markers for their trace detection in complex matrices [1]. Trace detection is achieved currently with optimal sensitivity and specificity using gas chromatography-tandem mass spectrometry (GC-MS/MS) [2–7]. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) (with electrospray ionization) has considerable potential in simplifying analyses of muramic acid [8,9] and other carbohydrates [10–13] since time-consuming derivatization reactions are avoided. Unfortunately, at this time, LC-MS/MS analysis of native sugars does not match the sensitivity of GC-MS/MS for derivatized sugars [3,9]. It has also been noted that LC-MS systems are considered much less user-friendly compared to their capillary GC-MS counterparts for environmental chemical analysis [14]. With GC-MS/MS assays, bacterial components, whether derived from viable or non-viable organisms or their constituents, are detected. Conventional microbiological culture is a far more widely used technique. However, an important portion of the bacterial population may remain undetected [7,15].

Muramic acid (3-O-lactyl glucosamine) is one of the few chemical markers that is unique to bacteria but widely distributed among bacterial species. Muramic acid is not generally found elsewhere in nature including animal cells and body fluids or fungi [2,4]. Thus mu-

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Dedicated to J.F.J. Todd and R.E. March in recognition of their original contributions to quadrupole ion trap mass spectrometry.

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ramic acid serves as a qualitative marker for the presence of peptidoglycan and muramic acid levels reflect the levels of peptidoglycan in quantitative assays [2–4].

High resolution chromatographic separations coupled with selective clean-up steps are important in improving the specificity of detection of chemical markers (e.g. muramic acid) in complex matrices. However, chromatographic separation is not sufficient to eliminate extraneous peaks when nonselective detectors are employed. The use of the mass spectrometer, as a selective GC detector [i.e. GC-MS analysis in the selected ion monitoring (SIM) mode], helps greatly in diminishing background noise. However, even using SIM it is not uncommon to find extraneous peaks [16,17]. The specificity of the tandem mass spectrometer in multiple reaction monitoring (MRM) mode as a GC detector provides even further specificity in detection at trace levels in complex matrices [2,3]. Both SIM GC-MS and MRM GC-MS/MS analysis allow excellent quantitation of chemical markers for bacteria but the latter provides greater confidence in assignment of peaks as the compound of interest by avoiding interferences [2].

When present at relatively high levels it is possible to categorically identify muramic acid in a chromatographic peak by the "total ion mass spectrum" (GC-MS analysis). For example, using GC-MS (after systemic administration of streptococcal cell wall components) a peak at the retention time for muramic acid found in rat spleen (70 μg/g wet weight of tissue) had an identical "mass spectrum" to that of standard muramic acid [16]. In samples prepared from joints of cell wall injected rats, a peak was observed, at 1 μg/g levels using SIM, at the retention time for muramic acid. However, a peak, was not observed (when joints from animals not injected with bacterial components, used as negative controls) were analyzed. However, at the low levels present in these biological samples it proved impossible to obtain a full mass spectrum for "absolute" identification using GC-MS [18].

Categorical identification at trace levels has awaited the development of more advanced GC-MS/MS instrumentation. Ion trap GC-MS/MS has been used for absolute identification at trace levels of muramic acid in human body fluids [4]. This is the only report to date using GC-MS/MS to detect muramic acid or indeed any other marker for bacteria in a human/animal body fluid or tissue. Product ion mass spectra (upon MS/MS analysis) of muramic acid peaks ($\geq$30 ng/mL) found in infected human body fluids were identical to those of pure muramic acid.

The first use of GC-MS/MS for trace detection of a chemical marker for bacteria, employed a triple quadrupole mass spectrometer and allowed the quantitative analysis of muramic acid, as it's alditol acetate derivative, in organic dust [2]. Subsequently successful use of more modestly priced and user friendly ion trap MS-MS instruments in this laboratory [3] and one other [5] have demonstrated the potential for expanding the use of GC-MS/MS outside of a few specialist analytical microbiology laboratories.

In summary, both triple quadrupole and ion trap GC-MS/MS instruments allow successful trace analysis of muramic acid in environmental and clinical samples. However, there has been no direct comparison of the two instrumental configurations for trace analysis of muramic acid (or indeed other bacterial compound) in such complex matrices. The present report summarizes the use of ion trap and triple quadrupole GC-MS/MS instruments in "identification" (using product ion spectra) or quantitation (MRM mode) for muramic acid analysis. In trace analysis, ion trap GC-MS/MS has been widely used (during the past years) for non-microbiological applications [19–25]. However, direct comparisons with the triple quadrupole are not generally performed in such studies. Comparisons, with the triple quadrupole, have been made using an earlier generation of research-grade ion traps [26]. The work presented here is thus likely to have relevance to trace analysis of many other compounds, of nonmicrobiological as well as microbiological origin, in a wide variety of biological matrices.

2. Experimental methods

Development of the alditol acetate derivatization procedure for analysis of muramic acid and other bacterial sugars in complex matrices has been reviewed in detail elsewhere [27,28]. The application of

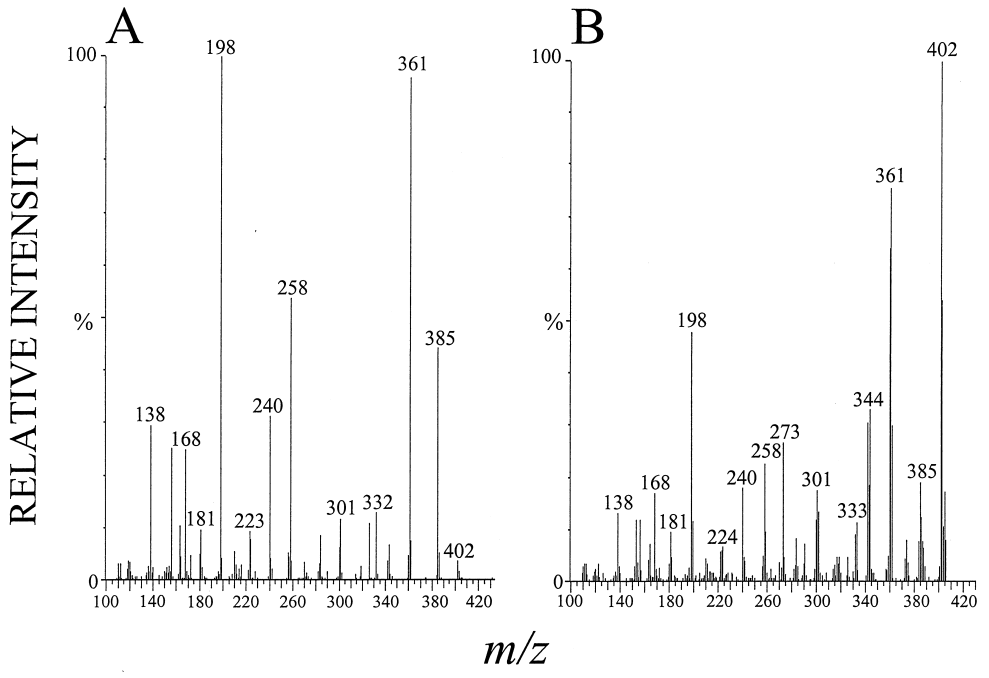

Fig. 1. Product ion spectra of alditol acetates of muramic acid. (A) standard (B) released by hydrolysis from an infected human body fluid. In each case, product ion spectra were obtained using an ion trap GC-MS/MS instrument. The fluid contained approximately 30 ng/mL muramic acid and 0.4 mL was analyzed. Reprinted from [4] with permission.

this procedure to GC-MS/MS analysis has also been described [2,3]. In brief, hydrolysis was performed for 3 h at 100 °C to release muramic acid from bacterial cell wall polymers. In quantitative studies, $^{13}CO_2$ labeled blue green algae (Isotec, Miamisburg, Ohio) were hydrolyzed and added as a source of ^{13}C muramic acid. These cyanobacteria were approximately 0.4% muramic acid on a dry weight basis. External standards consisted of a mixture of muramic acid and ^{13}C muramic acid derived from an algal hydrolysate. Samples were neutralized by mixing with *N*,*N*-dioctylmethylamine: chloroform (50:50 vol/vol). The aqueous phase was passed through a C-18 column (Analytichem, Harbor City, CA) and reduced with 5 mg sodium borohydride. To remove generated borate, methanol-acetic acid (200:1 vol/vol) was added continuously while evaporating under nitrogen. The samples were dried under vacuum. The alditols were acetylated at 100 °C overnight. Acetic anhydride was decomposed with 0.75 mL of water. One mL of chloroform was added and after mixing the aqueous phase discarded. 0.8 mL of ammonium hydroxide: water (80:20 vol/vol) was added and the mixture was passed through a Chem Elut column (Analytichem) and the chloroform phase collected. The samples were evaporated to dryness and resuspended in 30 μL of chloroform.

The ion trap MS-MS used in these studies was a GCQ (Finnigan, Atlanta, GA) and the triple quadrupole was a Quattro 1 (Micromass, Boston, MA). Both GC-MS/MS instruments were equipped with an automated sample injector (an A200S) and a nonpolar DB-5MS fused-silica capillary column (JW Scientific, Folsom, CA). Electron impact (EI) ionization was performed followed by collision induced dissociation (CID) of a precursor ion *m/z 403* (M-42, loss of ketene). Muramic acid and ^{13}C muramic acid were detected using the ion transitions *m/z 403* $\rightarrow$ *198* and *m/z 412* $\rightarrow$ *205*, respectively. Quantitation was based on peak area ratio of the two ion transitions in dust

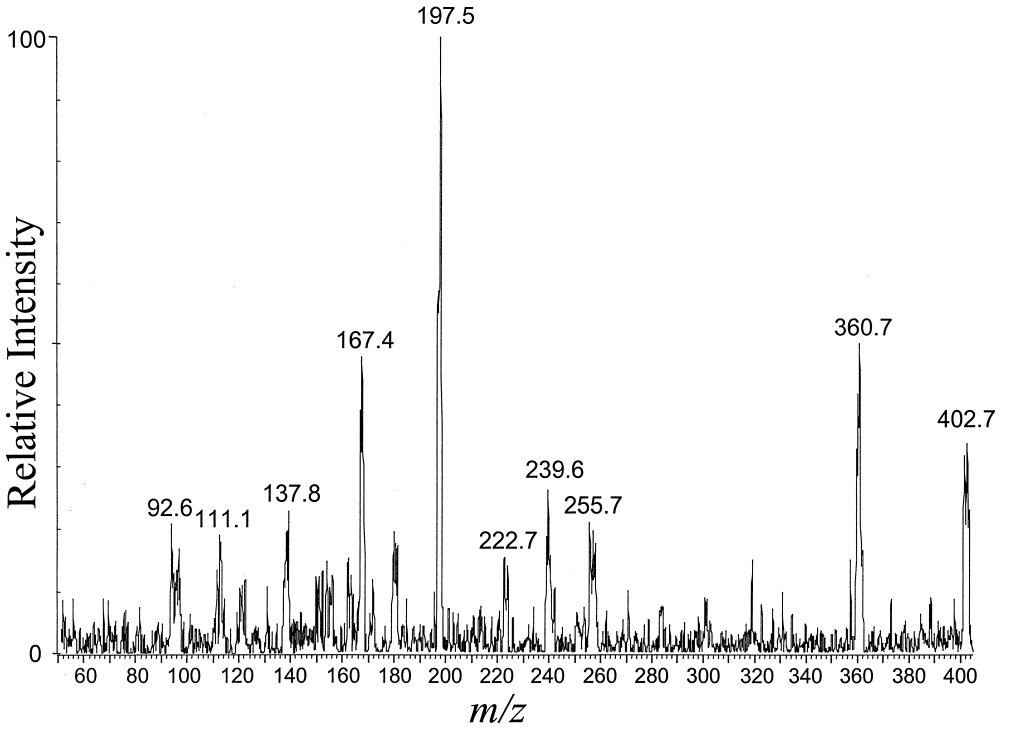

Fig. 2. Product ion spectrum of a muramic acid standard (320 ng derivatized and 32 ng instrumentally analyzed). The spectrum was obtained using a triple quadrupole mass spectrometer.

compared to the ratio in the external standard mixture (containing a known amount of muramic acid and ^{13}C muramic acid). Product ion spectra were of the dominant precursor ion (m/z 403).

Optimal sensitivity in MS/MS analysis requires that the precursor ion be present in relatively high abundance. In MRM analysis, selection of an ion with a higher mass-to-charge ratio may also contribute to lower background and product ion spectra are structurally informative in confirmation of compound identity [24]. Using the ion trap under automatic tune conditions, the mass spectrum of the alditol acetate of muramic acid is dominated by a high mass ion *m/z 403* (M-42, ketene) which was not the case for the triple quadrupole. To increase the abundance of the ion at *m/z 403* in triple quadrupole analysis ionization, a rhenium ribbon (generally used for chemical ionization) was used, filament voltage was set at 50 eV, source emission current at 200 μA and source temperature at 150 °C.

3. Results

Absolute identification of muramic acid in clinical matrices is readily achieved with the product ion spectrum. Muramic acid is found in infected body fluids but not aseptic controls. A typical product ion spectrum of muramic acid (after hydrolytic release and conversion to an alditol acetate derivative) detected in joint fluids from patients with septic arthritis is shown here to illustrate the power of ion trap GC-MS/MS for analysis of clinical specimens. Product ion spectra obtained from chromatographic peaks of pure muramic acid were identical (see Fig. 1). Muramic acid was detected at levels as low as 30

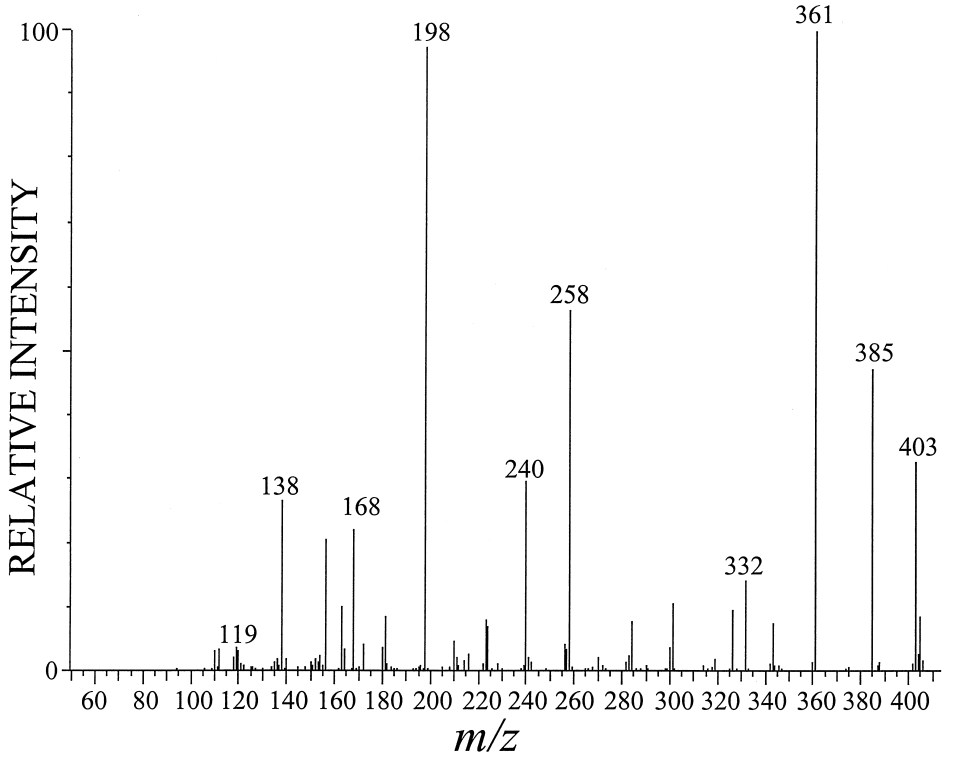

Fig. 3. Product ion spectrum of a muramic acid standard (320 ng derivatized and 32 ng instrumentally analyzed). The spectrum was obtained using an ion trap mass spectrometer.

ng/mL of (a total of 12 ng in 0.4 mL of human body fluid analyzed). The molecular weight of the alditol acetate of muramic acid (muramicitol pentacetate lactam) is 445, and loss of ketene generates an ion of *m/z* 403 in "normal" mass spectra. Product ion spectra of *m/z* 403 include *m/z* 361 from another loss of ketene, breakage at C4-C5 leads to loss of 145 generating a product ion of *m/z* 258; and the loss of acetic acid generates an ion of *m/z* 198. More details in the interpretation of these spectra may be found elsewhere [3].

The relative sensitivities of ion trap and triple quadrupole MS/MS instruments (product ion spectrum mode) are illustrated in the following example. Muramic acid (320 ng) was derivatized and two aliquots of the same sample (32 ng) were analyzed by GC-MS/MS (see Figs. 2 and 3 for examples of spectra obtained with ion trap and triple quadrupole analyses, respectively). The signal-to-noise ratios for a *m/z 198*-extracted ion were 75 for the ion trap and 9 for the triple quadrupole. At this level instrumental optimization for detection of muramic acid using the triple quadrupole instrument was demanding. In contrast 10 ng of derivatized muramic acid (1 ng analyzed) was readily detected using ion trap GC-MS/MS. Categorical identification of trace levels of muramic acid in complex matrices is a powerful feature of GC ion trap-MS-MS analysis.

In environmental matrices, such as soil and air, bacteria are commonplace and thus muramic acid, a bacterial component, is also ubiquitous. Determination of the mere presence of muramic acid as indicated by the product ion spectrum is of limited utility. In contrast, the levels of muramic acid are a useful indicator of biocontamination. Levels of muramic acid present in particulate dust collected from air by

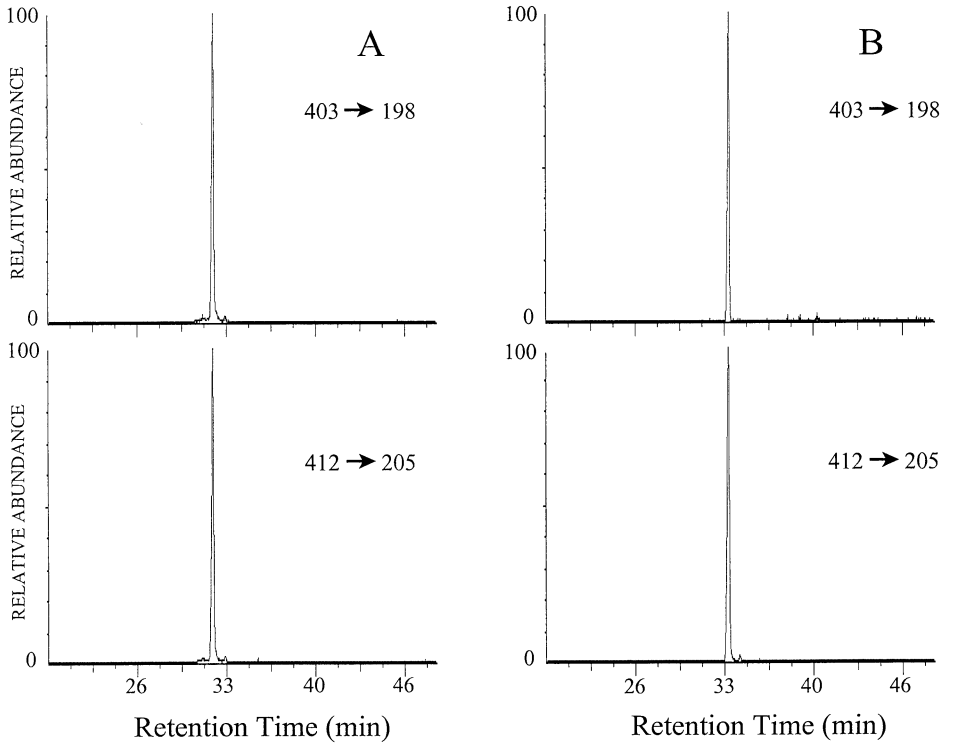

Fig. 4. MRM chromatogram of muramic acid derivative (A) standard (B) released by hydrolysis from airborne dust collected on a Teflon filter. The analysis was performed using an ion trap mass spectrometer.

filtration (ng/m^3 range) as well as in surface dust (ng/mg range) are readily quantitated using GC-MS/MS in MRM mode. As noted above, both ion trap and triple quadrupole instruments have been used successfully for this purpose. Figs. 4 and 5 show typical MRM chromatograms of muramic acid isolated from dust; using ion trap and triple quadrupole instruments, respectively. Chromatograms essentially free of background peaks are generated in both cases.

Varying amounts of pure muramic acid (0, 10, 20, 30, 40, 80, 160, 320, 640 ng) mixed with a constant amount of internal standard (^{13}C muramic acid) were derivatized. Samples were divided evenly and 1/10th of the sample injected into the two GC-MS/MS instruments. Analysis was performed in MRM mode and the ratio of ion transitions for muramic acid to ^{13}C muramic acid versus total starting muramic acid were plotted. Linearity was excellent for both instruments; triple quadrupole ($R^2 = 0.9992$) and the ion trap ($R^2 = 0.9779$) (see Figs. 6 and 7). Relative standard deviations for replicate analyses of the same sample (five injections total) and of multiple samples (five derivatized independently in a batch) were also of similar magnitude regardless of which instrument was used. Thus, sample preparation contributed minimally to the precision of the analysis. However, relative standard deviations were substantially higher for the ion trap versus the triple quadrupole (see Table 1).

In a recent comparison of quadrupole and ion trap instruments, in SIM mode, it was observed that the former is tenfold more precise in measuring ion ratios. Ions are collected by the trap, the ion of interest is then isolated by ejection of unwanted ions and scanned out for detection. In contrast, for the quadrupole instrument, ions are separated continuously by

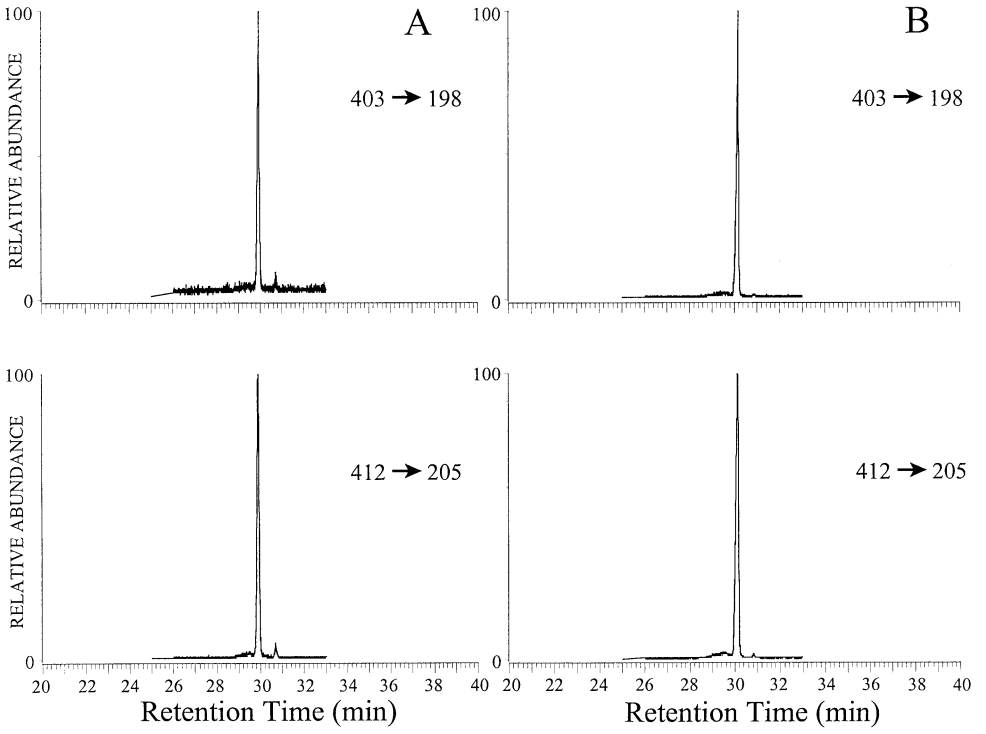

Fig. 5. MRM chromatogram of muramic acid derivative (A) standard (B) released by hydrolysis from surface dust. The analysis was performed using a triple quadrupole mass spectrometer.

the quadrupole before reaching the detector. Thus, for the ion trap, the possible number of scans in a given time period is reduced. This may limit the precision of the analysis [29]. In MRM, after trapping there is an additional step of excitation, to achieve CID before ions are ejected for detection [19]. In contrast, in the triple quadrupole instrument, ions are fragmented as they pass through the collision cell and product ions separated in the final quadrupole before reaching the detector. Thus, in MRM analysis there may be a

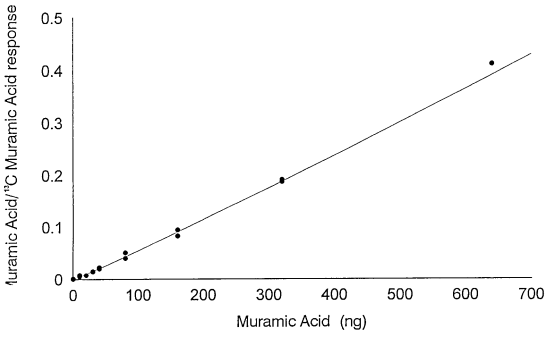

Fig. 6. Standard curve of MRM for muramic acid as its alditol acetate demonstrating linearity for the ion trap mass spectrometer ($R^2 = 0.9979$).

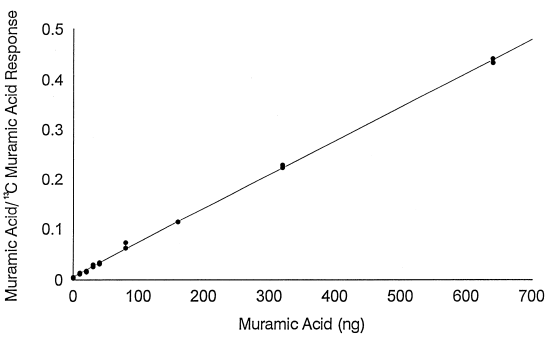

Fig. 7. Standard curve of MRM for muramic acid as its alditol acetate demonstrating linearity for the triple quadrupole mass spectrometer ($R^2 = 0.9992$).

Table 1
Quantitative analysis of muramic acid in multiple reaction monitoring mode using triple quadrupole and ion trap mass spectrometers

	Replicate injections		Multiple samples	
	Triple quadrupole	Ion trap	Triple quadrupole	Ion trap
Muramic acid (50 ng)	4.8%	17.8%	4.4%	19.4%
Muramic acid (500 ng)	1.6%	13.4%	2.2%	12.6%
Muramic acid from dust	1.6%	11.3%	6.2%	12.1%

further reduction in scans in a given time period, further affecting precision.

4. Conclusions

Direct comparisons of qualitative (product ion spectrum) and quantitative analyses (MRM mode) using the ion trap and triple quadrupole have not been widely reported. Sensitivity of the ion trap for absolute identification of muramic acid (product ion spectra mode) substantially exceeds that of the triple quadrupole. This might be anticipated based on theoretical considerations of instrument design ("tandem in time" versus "tandem in space") [26]. However, it is important that this has been experimentally demonstrated in trace detection in a variety of complex clinical and environmental matrices. It has been noted that ion trap detectors in general offer higher sensitivities in full scan mode than quadrupole mass spectrometers but when analyzing "real samples" the value of the spectral information is often limited by interference of matrix ions [14].

The utility of the product ion spectrum for absolute identification at trace levels in complex matrices, which is readily obtained with the ion trap, is a powerful feature. This is likely to have particular utility for studies of clinical specimens to determine the presence of bacteria (that are difficult to culture) or their nonviable cell wall components (not detectable by culture). Sterile body fluids and tissues (from healthy humans or animals) do not generally contain muramic acid. MRM analysis has utility for determining the levels of bacterial contamination for clinical and environmental analyses. Muramic acid levels have been demonstrated to serve as a useful measure of biocontamination of air [2,3,6,15]. For clinical and environmental applications, the precision obtained with the ion trap would more than suffice. Although, under the conditions employed in this study, reproducibility in quantitative analysis was substantially better using the triple quadrupole mass spectrometer. In summary, ion trap and triple quadrupole GC-MS/MS instruments have unique and complementary capabilities, respectively, in qualitative and quantitative analysis of muramic acid (and probably many other compounds) in complex samples. However, the low cost and ease of use of the ion trap makes it extremely attractive for diagnostic applications.

Acknowledgement

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