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Parent and Neutral Loss Monitoring on a Quadrupole Ion Trap Mass Spectrometer: Screening of Acylcarnitines in Complex Mixtures

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A novel and practical technique for performing both parent and neutral loss (P&NL) monitoring experiments on a quadrupole ion trap mass spectrometer is presented. This technique is capable of performing scans analogous to the parent and neutral loss scans routinely applied on tandem-in-space instruments and allows for the screening of a sample to detect analytes of a specific compound class on a chromatographic time-scale. Acylcarnitines were chosen as the model compound class to demonstrate the analytical utility of P&NL monitoring because of their amenability to electrospray ionization (ESI), their unique and informative MS/MS fragmentation pattern, and their importance in biological functions. The $[M + H]^+$ ions of all acylcarnitines dissociate to produce neutral losses of 59 and 161 amu and common product ions at *m*/*z* 60, 85, and 144. Both the neutral loss monitoring of 59 amu and the parent ion monitoring of *m*/*z* 85 are shown to be capable of identifying acylcarnitine $[M + H]^+$ ions in a synthetic mixture and spiked pig plasma. The neutral loss monitoring of 59 amu is successful in detecting acylcarnitines in an unspiked pig plasma sample.

Tandem mass spectrometers have found widespread use in analytical chemistry because of the sensitivity and selectivity provided by two (or more) stages of mass spectrometry (MS/MS and MS^n).[1,2] Traditionally, MS/MS has been performed on tandem-in-space instruments, such as triple quadrupole (TQMS) and multiple sector mass spectrometers.[3,4] More recently, tandem-in-time instruments, in which the stages of mass spectrometry are performed sequentially, as in the quadrupole ion trap mass spectrometer (QITMS)[5−7] and the Fourier transform ion cyclotron resonance mass spectrometer (FTMS),[8] have become more common.

The four most common MS/MS experiments are product ion scan, parent ion scan, neutral loss scan, and selected reaction monitoring (SRM). The most common of these experiments is the product ion scan, which acquires a mass spectrum of the product, or daughter, ions produced from the fragmentation of a selected parent, or precursor, ion. Inherently, product ion scans can be performed on both tandem-in-space and tandem-in-time instruments. A parent ion scan detects all of the parent ions in a sample that fragment to produce a common product ion, whereas a neutral loss scan detects those parent ions that fragment to produce product ions with a common difference in *m*/*z*, therefore losing a specific neutral fragment. Parent and neutral loss scans are important mass spectrometric techniques for mixture analysis and the screening of samples for the presence of specific compound classes.[1] These scans are readily implemented on tandem-in-space instruments, but are not readily implemented on tandem-in-time instruments.[7] The only direct approach to obtain this type of data on a tandem-in-time instrument is to acquire a series of separate product ion spectra for each parent ion. Each product ion spectrum requires a separate set of ionization, isolation, fragmentation, and mass analysis steps. Although software has been developed to "mine" out parent and neutral loss data from a series of product ion spectra on a commercial ion trap instrument,[9] it is highly time-intensive and cannot be accomplished on a chromatographic time scale. Two different approaches to obtaining parent and neutral loss scans on a QITMS have been previously developed in our lab,[10−12] but these approaches did not yield a practical method for implementation.

In this paper, a novel and practical technique for performing both parent and neutral loss (P&NL) monitoring experiments on a QITMS[13] is presented. This technique is capable of performing scans analogous to the parent and neutral loss scans routinely applied on tandem-in-space instruments and allows for the screening of a sample to detect analytes of a specific compound class

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on a chromatographic time-scale. Herein, both the functional capability and the analytical utility of P&NL monitoring will be demonstrated and discussed. The $[M + H]^+$ ions formed from Ultramark 1621, a mixture of fluorinated phosphazines, were chosen to demonstrate the function of P&NL monitoring because of their well-characterized ESI properties and unique CID fragmentation patterns.

Acylcarnitines were chosen as the model compound class to demonstrate the analytical utility of these techniques because of their amenability to ESI, their unique and informative MS/MS fragmentation pattern, and their importance in biological functions. Carnitine and acylcarnitines participate in a wide spectrum of biological functions, including the metabolism of fatty acids.[14] The presence of abnormally high levels of acylcarnitines in a patient's urine and plasma is indicative of a wide variety of inborn errors of metabolism.[15−17] The typical concentration of total short-chain and medium chain acylcarnitines is 6−10 nmol/mL of plasma in healthy patients and can be 2−3 times higher in patients with metabolic disorders.[18] Over the past decade, a large amount of effort has been invested to develop methods utilizing MS/MS to screen patients, specifically newborns, for inborn errors of metabolism related to abnormal acylcarnitine concentrations in blood (both red blood cells and plasma), urine, and tissue.[18−22] Because of the reliance on both parent and neutral loss scans for these screening methods, the triple quadrupole mass spectrometer has been the instrument of choice for these methods. In this paper, the applicability of P&NL monitoring on a QITMS is demonstrated for screening physiological samples to detect the presence of acylcarnitines.

EXPERIMENTAL SECTION

Instrumentation. All experiments were performed on the Finnigan LCQ (San Jose, CA), a commercial benchtop ESI-QITMS instrument. The LCQ applies a resonant ejection frequency at a q_z of 0.90 to facilitate mass analysis. For all MS/MS experiments, the parent ion of interest is moved to a q_z of 0.25, where a resonant excitation waveform is applied to fragment the parent ion through collision-induced dissociation (CID) with the He atoms in the ion trap. The energy applied to invoke CID is specified as a collision energy percentage of the total 5 V_{p-p}. The programs for P&NL monitoring were written in the ion trap control language (ITCL). All experiments were performed in positive ion mode. The normal scan speed of 0.18 ms/amu was employed for all experiments. All samples were infused into the mass spectrometer at a flow rate of 3 μL/min using the LCQ syringe pump. Each spectrum presented in this paper is the average of either 10 or 25 summed scans, where each summed scan is an average of 3 individual scans, termed microscans on the LCQ.

Samples. To prove the functional capability of P&NL monitoring, the ions of Ultramark 1621 (PCR, Gainesville, FL) were

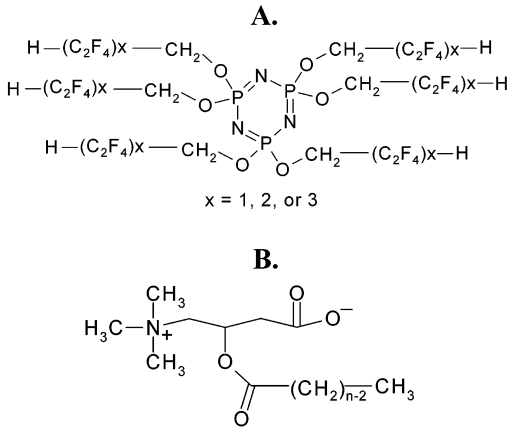

Figure 1. Structures of Ultramark 1621 and acylcarnitine compounds: (A) structures of Ultramark 1621 compounds from MW 921 to 2221, which increase by 100 amu with each additional C_2F_4; (B) structures for the acylcarnitine class of compounds, where n = the number of carbons in the ester chain. In solution, these compounds form a zwitterion.

investigated. Ultramark 1621 is present in the LCQ tuning solution, a mixture of 0.1 mM caffeine, 0.015 mM MRFA, and 0.3 mM Ultramark 1621 in a 50% acetonitrile (ACN)/25% methanol (MeOH)/25% H_2O solution (v/v), with 0.01% glacial acetic acid. All solvents were HPLC grade. Ultramark 1621 is a mixture of fluorinated phosphazines having molecular weights of 921 to 2221 amu at intervals of 100 amu. The structure of the fluorinated phosphazines is presented in Figure 1A. For this study, the ions of interest are the $[M + H]^+$ ions of Ultramark 1621, at m/z 922 to 1922 in 100-amu intervals.

To demonstrate the analytical utility of P&NL monitoring, a series of samples containing acylcarnitines was employed. For initial studies, a synthetic mixture of nine saturated acylcarnitines (CMIX) was analyzed. The components of CMIX are acetyl(C_2)-L-carnitine chloride (MW 239.7), propionyl(C_3)-L-carnitine chloride (MW 253.7), isobutryl(C_4)-L-carnitine chloride (MW 267.7), isovaleryl(C_5)-L-carnitine chloride (MW 281.8), hexanoyl(C_6)-L-carnitine chloride (MW 295.8), octanoyl(C_8)-L-carnitine chloride (MW 323.9), lauroyl(C_{12})-DL-carnitine chloride (MW 380.0), myristoyl(C_{14})-DL-carnitine chloride (MW 408.0), and palmitoyl(C_{16})-L-carnitine chloride (MW 436.1). Each acylcarnitine was purchased from Sigma Chemical (St. Louis, MO). The CMIX was initially prepared at a concentration of 3 mM for each acylcarnitine in a 50% MeOH/50% chloroform (v/v) solution. For ESI-MS analysis, the CMIX was diluted to 3 μM in 50% MeOH/50% H_2O (v/v) solution. The base chemical structure for each acylcarnitine is presented in Figure 1B. The identity and specifics of each acylcarnitine $[M + H]^+$ ion and its fragmentation data are displayed in Table 1.

Two physiological samples were investigated for the presence of acylcarnitines using P&NL monitoring. The first is a commercially available sample of pig plasma (Sigma-Aldrich, St. Louis, MO) that was spiked with 15 nmol/mL of each acylcarnitine (total concentration of 15 μM CMIX in the plasma) and then diluted by a factor of 10 in 50% HPLC-grade MeOH/50% HPLC-grade H_2O (v/v). The other sample was pig plasma obtained from a freshly slaughtered pig. Each was diluted by a factor of 5 in 50% HPLC-grade MeOH/50% HPLC-grade H_2O (v/v) for analysis.

Product ion mass spectra were acquired for the $[M + H]^+$ ions of the fluorinated phosphazines and acylcarnitines to determine the characteristic fragmentation pattern of each compound class.

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Table 1. Product Ion Data for Acylcarnitine $[M + H]^+$ Ions[a]

acyl (C_n) carnitine	$[M + H]^+$ (m/z)	$[M + H - 59]^+$ (m/z)	$[M + H - 161]^+$ (m/z)	common ions detected (m/z)	optimum collision energy (%)
acetyl (C_2)	204	145	ND[b]	60, 85	14.9
propionyl (C_3)	218	159	ND	85	15.3
isobutryl (C_4)	232	173	ND	85, 144	15.9
isovaleryl (C_5)	246	187	85	85, 144	17.0
hexanoyl (C_6)	260	201	99	85, 144	18.0
octanoyl (C_8)	288	229	127	85, 144	19.6
lauroyl (C_{12})	344	285	183	144	23.1
myristoyl (C_{14})	372	313	211	144	24.7
palmitoyl (C_{16})	400	341	239	144	26.6

[a] The detected product ions from each MS/MS experiment for the $[M + H]^+$ ions of each acylcarnitine present in the synthetic CMIX sample at the specified m/z. Both the product ions from characteristic neutral losses and common product ions are presented. The optimum collision energy, defined as the energy required to dissociate 95% of the parent ion, was determined for the dissociation of each $[M + H]^+$ ion. All product ion experiments were conducted at a resonant excitation q_z of 0.25. [b] ND = not detected.

Breakdown curves were constructed to determine the optimum and range of collision energies for each ion. The fragmentation patterns were utilized to determine characteristic neutral losses and common product ions for each ion in the compound class (Table 1). The breakdown curves were utilized to determine the optimum collision energy to use during P&NL monitoring experiments for the Ultramark and acylcarnitine ions. For the $[M + H]^+$ ions of Ultramark, a collision energy of 60% was used for all P&NL monitoring experiments. For the acylcarnitine ions, a collision energy percentage of 25% was used for all P&NL monitoring experiments. These collision energy percentages were chosen because they yielded structurally informative product ion spectra for each of the ions of interest.

Parent and Neutral Loss Monitoring. Programs consisting of a series of computer-controlled events composed in the ITCL for the LCQ were written for implementing P&NL monitoring on a QITMS. The series of computer-controlled events are the same for both parent ion monitoring and neutral loss monitoring, as shown in the flowchart in Figure 2; the only difference is the manner in which calculations pertaining to the target product ions are performed. In the case of the parent ion monitoring experiment, the detected product ion will remain constant for each parent ion interrogated. In the case of the neutral loss monitoring experiment, the detected product ion will be different for each parent ion interrogated and will be calculated from the neutral loss entered by the user. In this section, the computer-controlled events for P&NL monitoring will be described for both a prescan and an analytical scan. A pictorial representation of the process is presented in Figure 3.

First, ions may be either formed in the ion trap via internal ionization or injected into the quadrupole ion trap from an external ionization source. In the work presented here, an external electrospray ionization (ESI) source was utilized to form analyte ions for analysis. The injected ions are then mass-analyzed, and an initial mass spectrum, or prescan, is acquired. The computer can then select ions to be interrogated for either the parent ion or neutral loss monitoring experiment on the basis of a set of predetermined criteria. For the experiments described here, a m/z is selected for interrogation if it is one of the 18 most intense m/z ions in the acquired prescan. In Figure 3, the selected m/z ions are represented by the black closed circles. The number of parent ions that may be interrogated is restricted to 18 by the computer memory required for the method and not the P&NL monitoring method. To interrogate more than 18 parent ions and obtain a more "complete" P&NL spectrum, a series of P&NL

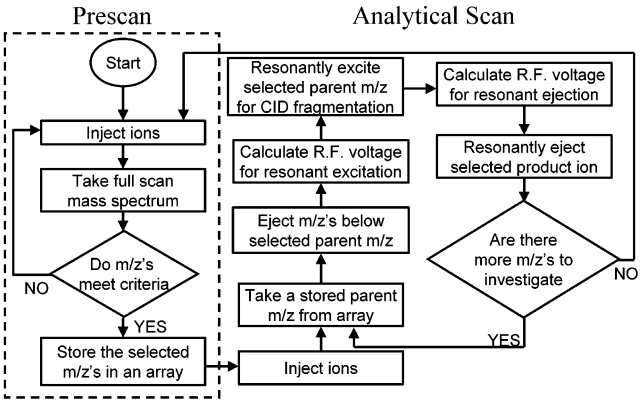

Figure 2. Flow chart for parent and neutral loss monitoring; the technique was performed on a quadrupole ion trap with both a prescan and analytical scan.

monitoring experiments may be performed in sequence utilizing multiple ionization events. For example, the first P&NL monitoring experiment may interrogate the 18 most intense ions and then the second experiment could interrogate the next most intense 18 ions. The general strategy for determining the criteria is to allow the computer to choose m/z values of the ions with enough intensity to produce good MS/MS spectra for analysis. Other possible criteria that may be utilized for selecting m/z ions to be interrogated include selecting ions from a predetermined list of ions or selecting the most intense ions that are not on an exclusion list. The selected m/z values are then stored in an array for interrogation.

Next, a second ionization event takes place, and all of the ions are trapped in the ion trap for analysis. The computer then selects the first (lowest) parent m/z that is to be interrogated. The RF amplitude is then ramped up until all of the ions of a m/z less than the selected m/z have a q_z value greater than 0.9 and are resonantly ejected from the ion trap. The resonant ejection waveform is applied during this event to facilitate ion ejection, but the electron multiplier is off, and no ion signal is acquired. This action is represented pictorially as step 1 in Figure 3.

After all of the ions of a m/z lower than the selected m/z have been ejected from the ion trap, the RF amplitude is ramped down to place the first selected parent m/z at a q_z value of 0.25. A resonant excitation waveform is then applied at a frequency corresponding to a q_z of 0.25, and the parent ion is fragmented

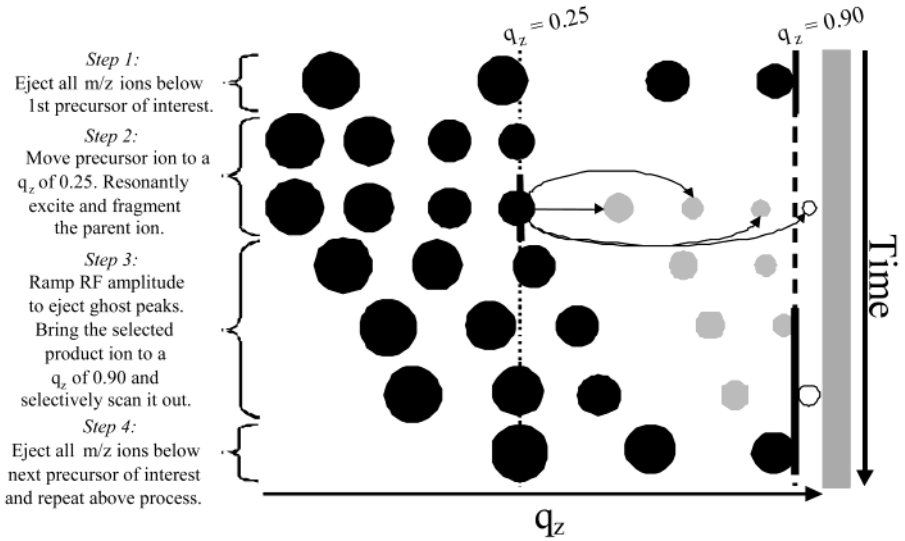

Figure 3. Illustration of parent and neutral loss monitoring, illustration of parent and neutral loss monitoring in q_z space along the $a_z = 0$ line. The shaded area on the right represents the edge of the stability diagram. The black circles represent parent ions, the gray circles represent product ions, and the open circles represent ions that have a q_z value >0.90. Failure to eject these ions will produce a false positive peak when the ions cross the 0.908 stability edge.

via CID. All of the product ions with a q_z value between 0.25 and 0.90 are then trapped. These actions are represented in step 2 in Figure 3.

After a sufficient time for parent ion fragmentation, typically 30 ms, and the product ions have been effectively trapped, the RF amplitude is quickly ramped up to place the targeted product m/z at a q_z of 0.90. The electron multiplier is off during this event. This action allows for all of the unwanted product ions to be ejected from the trap and not be detected. The targeted product m/z is then selectively ejected by ramping the RF amplitude with the electron multiplier on. If CID of the parent ion formed the targeted product ion, the ion will be detected. These actions are depicted in step 3 in Figure 3. The next step is to ramp the RF amplitude up until all of the ions of a m/z less than the second selected parent m/z are ejected from the ion trap. This action is represented in step 4 in Figure 3. The processes depicted in steps 1−4 are then repeated for each selected parent m/z until all of the selected m/z in the stored array are interrogated. An analytical scan is then assembled from the series of steps 1−4 (Figure 3) and displayed to the user, which depicts all of the selected m/z ions that fragment to produce the specified product ion (parent ion monitoring) or neutral loss (neutral loss monitoring).

For each P&NL ion experiment, a series of decisions must be made by the experimenter and specified as a set of parameters. The first decision is whether a parent ion monitoring or neutral loss monitoring experiment will be conducted. For a parent ion monitoring experiment, the product ion m/z to be monitored must be specified. For a neutral loss monitoring experiment, the neutral loss fragment must be specified so that the computer can calculate which product ion m/z should be monitored for each parent ion m/z. Parameters that must be entered include the parent ion m/z range to be interrogated, the number of m/z to be interrogated, the collision energy percentage for the CID experiments, and the width of isolation for the parent ions. Both the prescan and analytical scans can be individually averaged over the specified m/z range.

Other considerations for each P&NL monitoring experiment are the desired time scale and the number of individual scans that will be averaged for one P&NL monitoring scan. For a P&NL ion experiment that implements the 18 most intense ion criterion and has both a prescan and analytical scan over a 1500 m/z range, the time required for each individual scan is ~1.0 s. This includes ~300 ms for the prescan and ~700 ms for the analytical scan. The time for each scan will differ depending upon the selection criterion used, the number of ions interrogated, and the m/z range analyzed. The overall time to acquire an individual P&NL monitoring scan using this method is considerably shorter than if an entire product ion scan were acquired for each interrogated parent ion, since an entire product ion scan necessitates a scan over a large m/z range rather than over only a narrow m/z range.

RESULTS AND DISCUSSION

Ultramark 1621. To illustrate the functional capability of P&NL monitoring methods, the $[M + H]^+$ ions of Ultramark 1621 were used as a model system. To design an effective P&NL monitoring experiment for the detection of an Ultramark 1621 ion in a sample, the fragmentation pattern of the ions must be predetermined. To this end, CID experiments were performed on each Ultramark 1621 ion. It was determined that although there are some differences in the fragmentation patterns, all $[M + H]^+$ ions of Ultramark 1621 produce product ions corresponding to neutral losses of 32, 132, 232, 244, 332, 344, and 444 amu. Examples of product ion spectra for representative $[M + H]^+$ ions of Ultramark 1621 at m/z 1422 and m/z 1622, corresponding to 11 and 13 C_2F_4 groups, respectively, in its chemical structure, are presented in Figure 4A,B. It was determined that a collision energy of 60% is sufficient to cause some fragmentation for all of the Ultramark $[M + H]^+$ ions without ejecting any ions before fragmentation occurs.

The first step in either a neutral loss monitoring experiment or a parent ion monitoring experiment is to acquire a prescan of the sample. In this case, the prescan is a full-scan mass spectrum of the LCQ tuning solution from m/z 500 to m/z 2000 (Figure 5A). The ion at m/z 524 is the $[M + H]^+$ ion of the tetrapeptide MRFA, a component of the tuning solution. The positive ESI of the various fluorinated phosphazines in Ultramark 1621 produces intense $[M + H]^+$ ions at 100 amu intervals from m/z 922 to 1922. The m/z values that fit the predetermined criteria are then

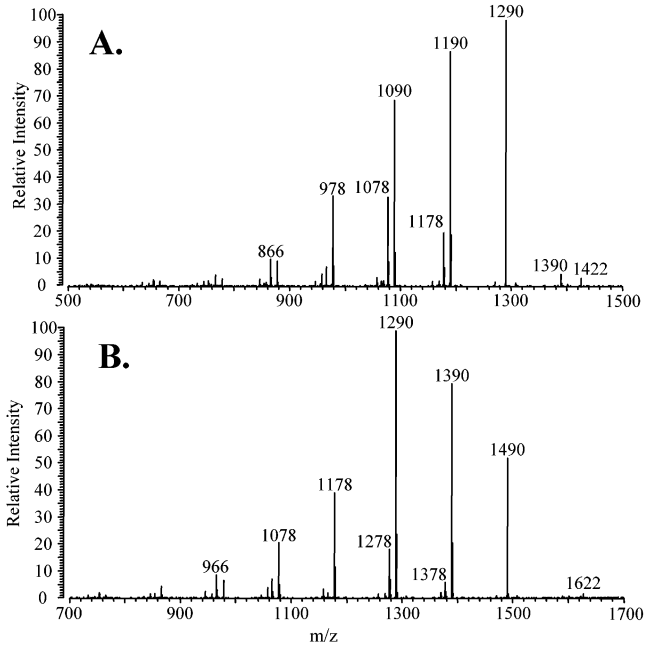

Figure 4. Product ion mass spectra of Ultramark $[M + H]^+$ ions: (A) product ion mass spectrum of $[M + H]^+$ at *m*/*z* 1422 and (B) product ion mass spectrum of $[M + H]^+$ at *m*/*z* 1622. Both spectra were obtained at a resonant excitation q_z of 0.25 and collision energy of 60%.

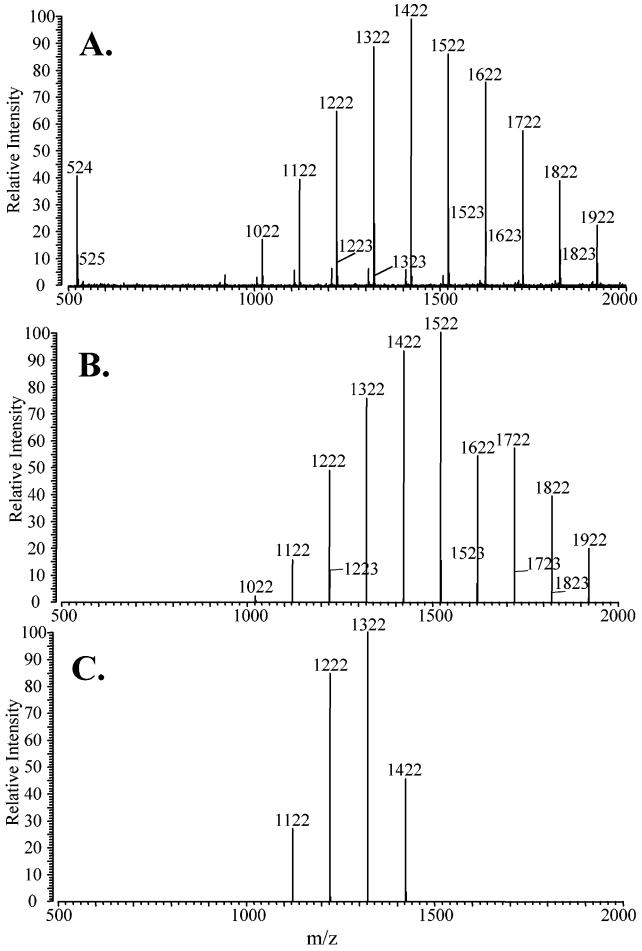

Figure 5. P&NL monitoring of LCQ tuning solution to screen for Ultramark $[M + H]^+$ ions: (A) prescan of LCQ tuning solution, (B) neutral loss monitoring of 132 amu, and (C) parent ion monitoring of *m*/*z* 1090. Both B and C were at an excitation q_z of 0.25 and collision energy of 60%.

selected and stored in an array for interrogation in the analytical scan. In this experiment, the criterion is that the ion is one of the 18 most intense in the prescan. Next, the second ionization event occurs, and ions corresponding to the stored *m*/*z* values are interrogated in order of increasing *m*/*z* to obtain the analytical scan for either a neutral loss or parent ion monitoring experiment.

In the product spectra of the $[M + H]^+$ ions of Ultramark 1621, the product ions resulting from the neutral loss of 132 amu were consistently intense. Therefore, a neutral loss of 132 amu was chosen as a characteristic neutral loss to screen for the $[M + H]^+$ ions of the fluorinated phosphazines. The neutral loss monitoring spectrum (Figure 5B) includes only the ions that are among the 18 most intense in the prescan (Figure 5B) and undergo a neutral loss of 132 amu. Using the 18 most intense ion criteria, all of the $[M + H]^+$ ions from *m*/*z* 1022 through 1922, plus five of the ^{13}C-peaks of these $[M + H]^+$ ions at one amu higher, were selected. All of these ions undergo a neutral loss of 132 amu. The other ions that were selected, including the $[M + H]^+$ ion of MRFA and the impurity ions 14 amu below the Ultramark $[M + H]+$ ions, do not fragment with a neutral loss of 132 amu and, therefore, do not show up in the neutral loss spectrum. The relative intensity of the ions in Figure 5B is a function of the parent ion intensity plus the efficiency of the CID process to form the particular product ion at the selected collision energy.

To design a parent ion monitoring experiment that will identify all of the Ultramark ions, a common product ion should be chosen. However, the $[M + H]^+$ ions do not all produce a common product ion under the CID conditions utilized for this experiment. At a collision energy of 60%, the $[M + H]^+$ ions at *m*/*z* 1122, 1222, 1322, and 1422 all produce a product ion at *m*/*z* 1090. However, the lower and higher *m*/*z* Ultramark $[M + H]^+$ ions, at *m*/*z* 922, 1022, 1522, 1622, 1722, 1822, and 1922, do not produce a product ion at *m*/*z* 1090. For our sample parent ion monitoring experiment, the product ion at *m*/*z* 1090 was chosen as the ion for which to screen. The parent ion monitoring spectrum (Figure 5C) detects only the four Ultramark $[M + H]^+$ ions in the prescan (Figure 5A) that fragment to produce a product ion at *m*/*z* 1090. If a parent ion monitoring experiment was needed that would detect all of the Ultramark 1621 $[M + H]^+$ ions, then more than one product ion would have to be selected.

Acylcarnitines. To demonstrate the analytical capability of P&NL monitoring for compound class screening, experiments were conducted utilizing acylcarnitines. CMIX, a synthetic mixture of nine acylcarnitines, was first analyzed to determine characteristic product ions and neutral losses. To evaluate P&NL monitoring on the ion trap, spiked and unspiked pig plasma samples were then analyzed.

To determine the fragmentation pattern of the $[M + H]^+$ ion for each acylcarnitine in the CMIX, product spectra were obtained for each ion. The characteristic product ions for the $[M + H]^+$ ions of each acylcarnitine at an excitation q_z of 0.25 are presented in Table 1. The acylcarnitine $[M + H]^+$ ions fragment to produce common product ions at *m*/*z* 60 ($[N(CH_3)_3 + H]^+$), 85 ($[CH_2{=}CHCHCO_2 + H]^+$), and 144 ($[N(CH_3)_3CH_2CHCHCO_2 + H]^+$). During an MS/MS experiment on a QITMS, the lowest *m*/*z* product ion that may detected, or low mass cutoff (LMCO), is determined by the parent *m*/*z*, the q_z of excitation, and the q_z for

ion ejection at either the right-hand edge of the stability diagram ($q_z = 0.908$), or the resonant ejection q_z (q_{ej}), as determined by the following equation:

$$\text{LMCO} = \frac{q_z}{q_{ej}}(m/z \text{ parent})$$

Since MS/MS excitation occurs at a default q_z of 0.25 and the q_{ej} is 0.90 on the LCQ, the product ion at m/z 60 can be detected only for acylcarnitines with a MW below 217 amu (i.e., only acetylcarnitine), and the product ion at m/z 85 can be detected only for acylcarnitines with a MW below 305 amu. By lowering the q_z of excitation to 0.16, the product ion at m/z 85 was detected from the dissociation of each acylcarnitine $[M + H]^+$ ion in the mixture. In addition, the product ion at m/z 60 was detected for all $[M + H]^+$ ions of the acylcarnitines m/z 288 and less. The common product ion at m/z 144, although observed for all of the acylcarnitines, is not a very intense product ion. Each acylcarnitine $[M + H]^+$ ion also produces a product ion that corresponds to a neutral loss of 59 amu, $N(CH_3)_3$, and 161 amu, $N(CH_3)_3CH_2CH(OH)CHCO_2H$. The neutral loss of 59 was detected for all acylcarnitine $[M + H]^+$ ions at an excitation q_z of 0.25. The product ion associated with the neutral loss of 161 could not be detected for the lowest MW acylcarnitines (acetylcarnitine, propionylcarnitine, and isobutrylcarnitine) at an excitation q_z of 0.25 because of the LMCO. To detect the neutral loss of 161 amu for the low-MW acylcarnitines, the q_z of excitation was decreased. The product ion corresponding to the neutral loss of 59 amu is consistently the most intense product ion. Breakdown curves were constructed for the $[M + H]^+$ ion of each acylcarnitine, and the optimum collision energy percentage was ascertained (Table 1). A collision energy percentage of 25% was found to be nearly optimum for the CID of each acylcarnitine ion.

Both neutral loss monitoring and parent ion monitoring experiments were conducted on the CMIX sample. In these experiments, the 18 most intense ion criterion was used for selection of the m/z to interrogate from a prescan (Figure 6A) over a m/z range of 100−600. The prescan is the average of the prescans from 20 successive P&NL monitoring experiments and is representative for both the neutral loss monitoring and parent ion monitoring experiments.

The spectrum obtained by neutral loss monitoring of 59 amu of CMIX is shown in Figure 6B. All of the acylcarnitine $[M + H]^+$ ions selected as one of the most 18 intense from the prescan were detected, including the ^{13}C $[M + H]^+$ ion of some acylcarnitines. Comparison of the prescan (Figure 6A) and the analytical scan (Figure 6B) shows that ions that were selected as among the 18 most intense, but are not acylcarnitine $[M + H]^+$ ions, such as the ion at m/z 156, are not observed in the neutral loss monitoring spectrum. Each neutral loss monitoring spectrum, consisting of 3 individual scans for both the prescan and analytical scan, required an average of 3.3 s to acquire.

Figure 6C shows the spectrum obtained by parent ion monitoring of m/z 85 for CMIX at an excitation q_z of 0.25. Each of the C_2−C_8 acylcarnitine $[M + H]^+$ ions was detected. The C_{12}−C_{16} acylcarnitine ions were not detected, because their m/z 85 product ions fell below the LMCO during resonant excitation at a q_z of 0.25. When designing a parent ion monitoring or neutral loss monitoring experiment on the ion trap, it is important that a common product ion for parent ion monitoring or a characteristic neutral loss for neutral loss monitoring that produces product ions above the LMCO for all members of the compound class of interest be selected.

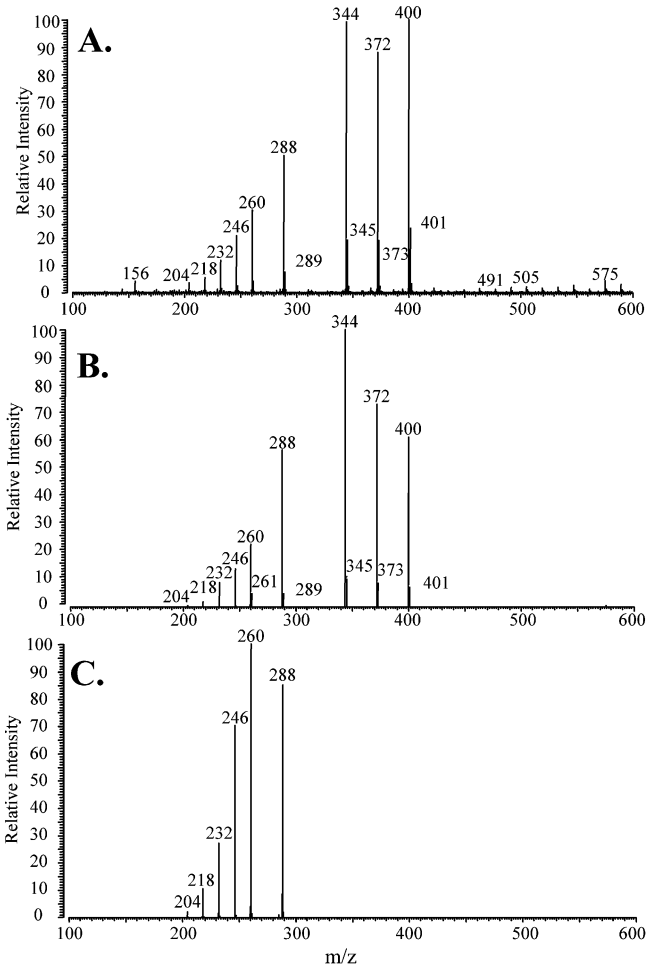

Figure 6. Parent and neutral loss monitoring of CMIX to screen for acylcarnitine $[M + H]^+$ ions: (A) prescan of CMIX solution, (B) neutral loss monitoring of 59 amu, and (C) parent ion monitoring of m/z 85. Both B and C were at an excitation q_z of 0.25 and collision energy of 60%.

Monitoring the neutral loss of 59 amu was selected as the best method for screening a sample for acylcarnitines, because these product ions are intense, and they can be monitored without any limitations associated with the LMCO. Note that parent monitoring of m/z 85 and neutral loss monitoring of 161 amu could be used as confirmation techniques for low- and high-MW acylcarnitines, respectively.

Spiked Pig Plasma. The spiked pig plasma was utilized to determine whether the P&NL monitoring methods would be capable of detecting acylcarnitines at physiologically relevant levels. Both neutral loss monitoring of 59 amu and parent ion monitoring of m/z 85 experiments were conducted on the sample, which was introduced via infusion. A prescan (Figure 7A) was acquired over a m/z range of 100−600. The prescan shows that the $[M + H]^+$ ions of all of the spiked acylcarnitines are detectable in the spiked sample, along with a variety of other ions, including the $[M + Na]^+$ ions for some of the spiked acylcarnitines. $[M + Na]^+$ ions were also detected in synthetic mixtures of acylcarnitines containing 10 mM sodium acetate. The fragmentation pattern for the $[M + Na]^+$ ions shows the neutral loss of 59, as for the $[M + H]^+$ ions, but not the neutral loss of 161 amu nor the common product ions at m/z 85 and 144.

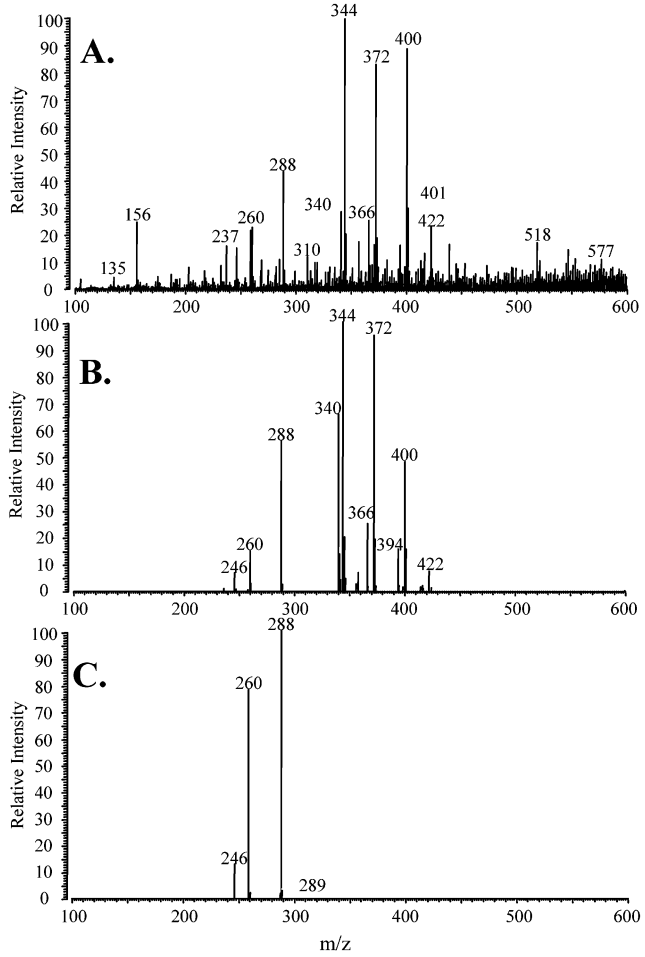

Figure 7. Parent and neutral loss monitoring of spiked plasma to screen for acylcarnitine $[M + H]^+$ ions: (A) prescan of spiked plasma with 15 μM CMIX, (B) neutral loss monitoring of 59 amu, and (C) parent ion monitoring of *m*/*z* 85. Both B and C were at an excitation q_z of 0.25 and collision energy of 60%.

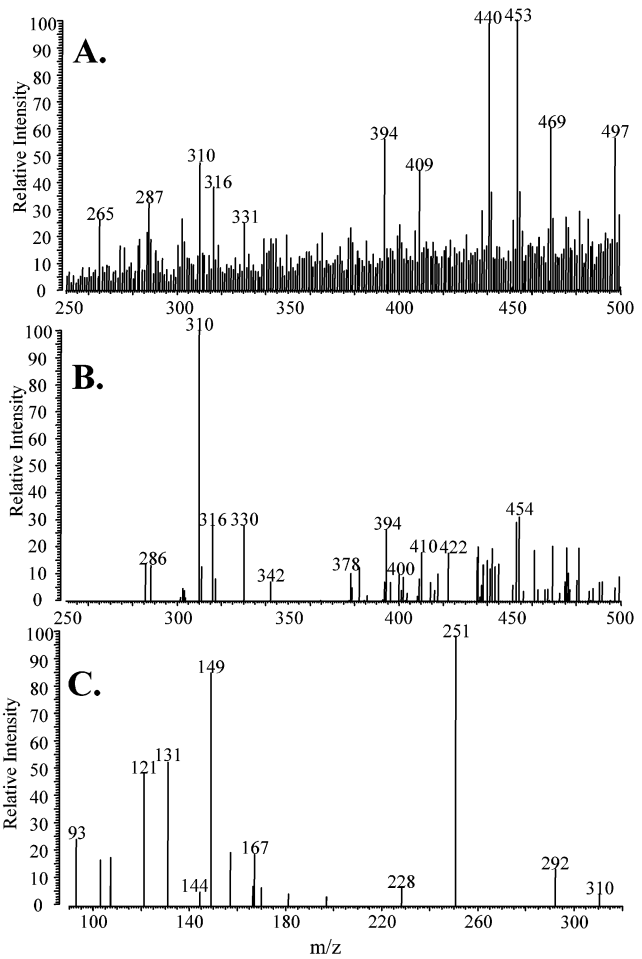

Figure 8. Neutral loss monitoring of pig plasma to screen for acylcarnitine $[M + H]^+$ ions: (A) prescan of an unspiked pig plasma sample, (B) neutral loss monitoring of 59 amu, and (C) product ion spectrum of *m*/*z* 310 from *m*/*z* 90 to 320.

Neutral loss monitoring of 59 amu for the spiked pig plasma sample (Figure 7B) shows the detection of many of the $[M + H]^+$ ions and $[M + Na]^+$ ions of the present acylcarnitines. The reason some acylcarnitine ions were not detected is that these ions were not selected as among the 18 most intense *m*/*z*, and thus, they were not interrogated. If it were of concern to detect these ions, a different criterion for selection of the *m*/*z* for interrogation could be used, or the second most intense set of 18 *m*/*z* could be selected for interrogation. This experiment detected only one ion, at *m*/*z* 340, in the spiked pig plasma sample that could not be attributable to the spiked acylcarnitines. A product ion spectrum of this ion confirmed that a product ion at *m*/*z* 281 was produced by CID, which corresponds to a neutral loss of 59 amu, but the product ion spectrum was not indicative of an acylcarnitine $[M + H]^+$ ion.

The parent ion monitoring of *m*/*z* 85 (Figure 7C) shows the detection of the $[M + H]^+$ ions for only isovalerylcarnitine, hexanoylcarnitine, and octanoylcarnitine in the sample. The higher MW acylcarnitines are not observed, because the product ion at *m*/*z* 85 is not detectable for parent ions above *m*/*z* 306 at an excitation q_z of 0.25. The lower MW acylcarnitines are not detected, since their $[M + H]^+$ ions are not among the 18 most intense *m*/*z*, and therefore, they were not selected for interrogation. There were no $[M + Na]^+$ ions detected in the sample, because those acylcarnitine ions do not produce a product ion at *m*/*z* 85.

Unspiked Pig Plasma. To demonstrate that this method is capable of detecting acylcarnitines in direct physiological samples, fresh pig plasma was obtained and analyzed. The analysis of this sample was performed with no prior knowledge about the presence of acylcarnitines in the sample. Neutral loss monitoring of 59 amu was used as the screening technique to determine the presence of acylcarnitines.

Acylcarnitines could be detected by neutral loss monitoring in the plasma sample. The mass spectrum in Figure 8A displays the average prescan from 15 consecutive neutral loss monitoring experiments of the plasma sample from *m*/*z* 250 to 500. For each prescan, the 18 most intense *m*/*z* were selected for interrogation. The selected *m*/*z* varied from scan to scan, depending on the intensity of each *m*/*z* for each prescan. Over the 15 prescans, a total of over 50 different ions were selected and interrogated for the neutral loss of 59 amu. The mass spectrum of the average of the 15 neutral loss monitoring experiments is displayed in Figure 8B. The mass spectrum shows all of the ions that have a neutral loss of 59 amu. Note that the NL of 59 is not specific for acylcarnitines. Confirmation of which of these ions actually corresponds to acylcarnitines can be performed by obtaining product ion spectra for each, as is commonly done in MS/MS compound-class screening.[23,24] Through inspection of the product

spectra for each of the detected ions, it appears that five of these ions arise from acylcarnitines.

In the unspiked plasma sample, we were able to detect the $[M + H]^+$ and $[M + Na]^+$ ions of palmitoylcarnitine (m/z 400 and 422), one of the acylcarnitines in the synthetic mixture for which we were looking in this sample. We were also able to identify acylcarnitine ions we had not previously observed, including m/z 310, 316, and 330. The product ion spectra for each were obtained; that for m/z 310 is presented in Figure 8C. The mass spectrum shows the common product ion at m/z 144 and the characteristic neutral losses of 59 and 161 amu (at m/z 251 and 149, respectively). In addition, the product ion at m/z 103 is common to some of the acylcarnitines. The assumed identity of the three ions is as follows: (1) the ion at m/z 310 is the $[M + H]^+$ ion of the C_9 acylcarnitine containing three double bonds in its acyl chain, (2) the ion at m/z 316 is the $[M + H]^+$ ion of the saturated C_9 acylcarnitine, and (3) the ion at m/z 330 is the $[M + H]^+$ ion of the saturated C_{10} acylcarnitine; none of these compounds was available as standards for confirmation.

CONCLUSION

The data presented here demonstrate that P&NL monitoring on the QITMS is a viable technique for screening analytical samples on a chromatographic time scale. This technique can yield valuable information, analogous to parent and neutral loss scans on tandem-in-space instruments, and compound-class screening of analytical samples.

Using both the spiked and unspiked pig plasma samples, the technique of neutral loss monitoring was demonstrated to be capable of detecting acylcarnitines in biological matrixes at physiological concentrations. In the spiked sample, the neutral loss monitoring of 59 amu detected both the $[M + H]^+$ and $[M + Na]^+$ ions of the spiked acylcarnitines. It also detected an ion at m/z 340 that had a neutral loss of 59 amu but was not identified as an acylcarnitine ion on the basis of its product spectrum. Parent ion monitoring of m/z 85 detected only a few acylcarnitines in the spiked pig plasma samples because of LMCO limitations and the low intensity of the $[M + H]^+$ ions for the low MW acylcarnitines, which resulted in their not being selected for interrogation. The neutral loss monitoring of 59 amu was successful in detecting acylcarnitines in an unspiked pig plasma sample. The identified acylcarnitines included those that were in the synthetic mixtures as well as those not in the synthetic mixture.

A few conclusions can be drawn concerning the application of P&NL monitoring for the detection of acylcarnitines in complex mixtures. First, the neutral loss monitoring of 59 amu is the most inclusive means to screen for acylcarnitines, because it will allow for the detection of all acylcarnitines, independent of MW or ion type ($[M + H]^+$ or $[M + Na]^+$). Next, it is important to minimize the potential for false negatives in the screening experiment due to a nonselection of the acylcarnitine ion for interrogation if it is not one of the most intense 18 ions in the prescan. To accomplish this goal, one can modify the selection criteria for the m/z to be interrogated. This could be accomplished by interrogating a set of the second most intense 18 ions, or if there is a specific and limited list of acylcarnitine ions that are of concern to the investigator, a "to-do" list can be created and only those ions will be interrogated by the analytical scan of the P&NL monitoring technique. Finally, because of the potential for false positives in the screening experiment, methods are required for the confirmation of the suspected acylcarnitine ion. This can be accomplished either by a complementary P&NL monitoring experiment, such as the neutral loss monitoring of 161 amu or the parent ion monitoring of either m/z 85 or 144, or by a product ion experiment. Ultimately, the technique of P&NL monitoring on the QITMS can be a valuable screening method for acylcarnitines.

An overall evaluation of P&NL monitoring experiments indicates that these methods can be useful in a variety of instances, including those involving multiply charged species. The key requirement of this method is that the product ion chosen for monitoring must have a lower m/z than the parent ion selected for interrogation. Such experiments would include the analysis of all singly charged ions, such as from small drug molecules and small peptides, as well as the monitoring of multiply charged parent ions for neutral losses and for product ions that are lower in m/z than the parent ion. In a neutral loss experiment, the group lost does not have a charge, so the charge state of the ion will not change, and the monitored ion will have a lower m/z than the parent ion. For example, this would include the monitoring of lost phosphate, sulfate, and carbohydrate groups from multiply charged, posttranslationally modified peptides. The only other requirement is that the m/z of the monitored ion be above the LMCO. In practice, this is not a significant limitation for neutral loss monitoring experiments, since the group lost is typically a small neutral, although it must be considered in the design of parent ion monitoring experiments.

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