Differences in the Low-Energy Collision-Activated Dissociation of Carboxylate Anions from Structurally Similar Prostaglandins: E_2 , $F_{2\alpha}$, D_2 , and $DHKE_{2\alpha}$

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Some intriguing discoveries were made concerning the collision-activated dissociation behavior of the derivatized carboxylate anions of PGE_2 and $PGF_{2\alpha}$. The carboxylate anion $[M-PFB]^-$ formed from electron-capture negative chemical ionization of the pentafluorobenzyl ester-trimethylsilyl derivative of $PGF_{2\alpha}$ showed little fragmentation under typical collision gas pressures and energies (<2.0 mtorr N_2 and <20 eV). In contrast, the daughter spectra of the carboxylate anion of the methoxime-pentafluorobenzyl ester-trimethylsilyl derivative of PGE_2 produced many intense fragments under the same conditions. (*J Am Soc Mass Spectrom* 1990, 1, 389-396)

Trace analysis of arachidonic acid metabolites such as prostaglandins continues to pose a challenging analytical problem [1]. Prostaglandin concentrations in biological fluids have been determined by bioassay, radioimmunoassay (RIA), high-performance liquid chromatography (HPLC), gas chromatography (GC), and gas chromatography combined with mass spectrometry (GC/MS). It has been shown that with these methods prostaglandin concentrations are often overestimated [2]. This problem can be traced to the lack of accuracy, reliability, and poor specificity of the techniques used for analysis.

The formation of a methoxime-pentafluorobenzyl ester-trimethylsilyl (MO-PFB-TMS) derivative followed by subsequent analysis with capillary GC electron-capture negative chemical ionization (EC-NCI) mass spectrometry is considered to be the most sensitive and selective technique in the analysis of prostaglandins [3-9]. However, selected-ion monitoring (SIM) traces sometimes contain many interfering peaks, thereby not allowing reliable quantitation. Gas chromatography combined with tandem mass spectrometry (GC/MS/MS) can help to diminish this problem through a reduction of the chemical noise utilizing the selected-reaction monitoring (SRM) mode [10, 11]. The ions formed by electron ionization (EI) of the methyl ester/methoxime trimethylsilyl ether derivatives of prostaglandins show considerable fragmentation in the collision-activated dissociation (CAD) pro-

cess [12, 13]. However, the carboxylate anions of certain prostaglandins produced by EC-NCI have been reported to be extremely stable when subjected to CAD [14].

It has been observed in our lab that the carboxylate anions of certain prostaglandins exhibit little fragmentation even at high collision energies (>20 eV) and pressures (1.5 mtorr N_2). We have found that the subtle differences among the structures of prostaglandins E_2 (PGE_2), $F_{2\alpha}$ ($PGF_{2\alpha}$), D_2 (PGD_2), and 13,14-Dihydro-15-keto $F_{2\alpha}$ ($DHKE_{2\alpha}$) (Figure 1) yield enormous differences in CAD efficiency. The CAD efficiency for the $[MOX/TMS-PFB]^-$, $[M-PFB]^-$, and $[M-H]^-$ carboxylate anions is significantly different for closely related prostaglandins. The low fragmentation and CAD efficiencies of the carboxylate anions of $PGF_{2\alpha}$ and $DHKE_{2\alpha}$ compared to those of PGE_2 and PGD_2 clearly indicate the greater stability of these species. Here we evaluate these differences and explain them in relation to the structural differences between the carboxylate anions for the prostaglandins.

Experimental

Prostaglandins and Reagents

The prostaglandins E_2 , $F_{2\alpha}$, D_2 , and 13,14-Dihydro-15-keto $F_{2\alpha}$, as well as O-methylhydroxylamine hydrochloride, N,N-diisopropylethylamine, pyridine, and acetonitrile (analytical grade) were all purchased from Sigma Chemical Co. (St. Louis, MO). Pentafluorobenzylbromide (PFBBr) and bis(trimethylsilyl)trifluoroacetamide (BSTFA) were purchased from Pierce Chemical Co. (Rockford, IL). The methane ($>99\%$) used as the chemical ionization reagent gas was purchased

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Figure 1. Structures of the four prostaglandins studied: (a) PGF_{2α}; (b) PGE₂; (c) PGD₂; (d) DHKF_{2α}.

Products, Inc. (Orlando, FL). Helium used as GC carrier gas and nitrogen used as CAD collision gas were commercial grade, with their purity checked by mass spectrometry.

Derivatization

The methoxime-pentafluorobenzyl ester-trimethylsilyl (MO-PFB-TMS) derivatives (Figure 2) were formed for the GC/MS/MS studies. The method used was similar to the derivatization of Hubbard et al. [15]. A 1-μg quantity of each prostaglandin was treated with 100–200 μL of methylhydroxylamine HCl in dry pyridine (4 mg/mL), allowed to stand overnight at room temperature, then evaporated under nitrogen until dry. Each sample was acidified by adding 200 μL of 1N formic acid, extracted with two 1 mL aliquots of ethyl acetate, and the extract dried under nitrogen. Then 50 μL of acetonitrile, 30 μL of 30% of PFBBr in acetonitrile, and 15 μL of 10% N,N-diisopropylethylamine in acetonitrile were added to the dried methoxime derivative. Each solution was allowed to stand for 30 min at room temperature before the reagents were evaporated with nitrogen. Excess derivatizing reagent was removed by dissolving

the sample in 200 μL of distilled water then extracting with two 1 mL aliquots of a methylene chloride:hexane (50:50 volume:volume); the extract was then dried under nitrogen. The trimethylsilyl derivative was formed by adding 100 μL of BSTFA and allowing to stand overnight at room temperature. Dilutions were made from this solution so that a 500 pg/μL solution of each prostaglandin was used for injections. The solids probe/MS/MS studies were performed either by analyzing the standards without derivatization or as the PFB derivative, using only the PFBBr esterification step above.

Sample Introduction

Gas chromatographic separation was carried out on a short J&W Scientific (Folsom, CA) DB-1 (3-m long, 0.25 mm i.d., 0.25 μm film thickness) capillary column in the splitless mode with helium carrier gas at an inlet pressure of 4–6 psi. The initial temperature of 200 °C was held for 30 s, then increased at 20 °C/min to 260 °C. The injector temperature was 300 °C. One-microliter injections of a 500-pg/μL solution of each prostaglandin were made in triplicate at each condition for the GC/MS/MS studies.

The solids probe was used as the means for sample introduction to study the PFB ester derivatives

Figure 2. Structures of the MO-PFB-TMS derivatives of the four prostaglandins: (a) PGF_{2α}; (b) PGE₂; (c) PGD₂; (d) DHKF_{2α}.

and the free (nonderivatized) prostaglandin standards. The initial temperature was 50 °C and increased at 20 °C/min to 300 °C. Triplicate samples were analyzed for each derivatization procedure at each condition for the MS/MS studies. Sample size was 1 µg of the non-derivatized prostaglandins or 1 ng of the PFB ester derivatives.

Tandem Mass Spectrometry

A Finnigan MAT TSQ45 (San Jose, CA) gas chromatograph/triple quadrupole mass spectrometer was employed. Mass spectrometry conditions were interface and transfer line temperature 300 °C, ionizer temperature 190 °C, electron energy 100 eV, and emission current 0.3 mA. Electron-capture negative chemical ionization was carried out with methane at an ionizer pressure of 0.45 torr.

In the MS/MS experiments, nitrogen collision gas pressure and collision energy were varied depending on each experiment. The [MOX/TMS-PFB]⁻ or [M-PFB]⁻ carboxylate anion was selected in the first quadrupole (Q1) region and passed into the collision cell (Q2). In this region these ions underwent CAD to form characteristic fragments that were then mass-analyzed in the third quadrupole (Q3) region. A full daughter spectrum was acquired over the mass range of 55-600 u. The maximum collision energy possible on the TSQ45 is 30 eV.

The peak areas in the daughter spectra of selected daughter ions and the parent ion remaining after CAD were calculated by the INCOS computer system for each GC and solids probe sample. A baseline was chosen visually and the calculated areas were used for determining CAD efficiencies.

Results and Discussion

The abundance of the daughter ions relative to that of the remaining parent carboxylate anion in the daughter spectrum can be controlled by varying the CAD energy or pressure; these parameters also affect sensitivity due to scattering losses. The processes of fragmentation and scattering can be monitored by evaluating the fragmentation (E_F), collection (E_C), and overall CAD (E_{CAD}) efficiencies given by the following equations [16]:

$$E_F = \frac{\sum D_i}{P + \sum D_i} = \text{fraction of ions present following CAD that are daughter ions}$$

$$E_C = \frac{P + \sum D_i}{P_0} = \text{fraction of initial parent ions that is collected following CAD as either parent or daughter ions}$$

$$E_{CAD} = \frac{\sum D_i}{P_0} = \text{fraction of initial parent ion that is converted to collectable daughter ions}$$

where P_0 , P , and, D_i are the intensities of the parent ion prior to CAD, the parent ion remaining after CAD, and a daughter ion resulting from CAD, respectively. Note that $E_{CAD} = E_F \times E_C$.

Monitoring the above processes can help in determining optimum MS/MS conditions for trace determination of prostaglandins. These efficiencies are affected by collision energy and collision gas pressure. Either parameter can be varied to maximize the CAD efficiency for a particular parent ion. Increasing the collision energy allows for more energetic collisions, while increasing the collision gas pressure increases the number of collisions each ion experiences. Either approach increases the amount of energy deposited into the parent ion, and thereby increases the fragmentation efficiency. However, an increase in collision energy or pressure will produce an increase in scattering losses (or possibly other loss mechanisms such as neutralization by charge exchange) and thereby decrease collection efficiency. The overall CAD efficiency, as the product of fragmentation and collection efficiency, will typically first increase then level off and even decrease as the collision energy or pressure is increased. Systematic variation of each parameter would provide a three-dimensional plot of efficiency versus energy versus pressure. Practically, such studies involve two-dimensional slices through this three-dimensional surface, varying one parameter while keeping the other constant.

Collision-activated Dissociation of the [MOX/TMS-PFB]⁻ Ions of Derivatized Prostaglandins

Pressure-resolved breakdown curves for selected ions of the PFB-TMS derivative of PGF_{2α} and the MO-PFB-TMS derivative of PGE₂ are shown in Figure 3. The optimum collision gas pressure is indicated on each curve. This type of curve can be calculated by dividing the area of a selected daughter ion by the area of all the ions in the daughter spectrum ($D_i / (\sum D_i + P)$) at each pressure. The point that is chosen as the optimum is at a pressure where one can obtain a qualitative daughter spectrum with a number of reasonably abundant daughter ions. Note that the optimum pressure for PGF_{2α} is five times higher than that for PGE₂.

Trace analysis by selected reaction monitoring with MS/MS requires optimization of the absolute intensity of a single daughter ion of the selected parent ion. The curves in Figure 4 give an indication of the optimum reactions and collision gas pressures that should be selected for maximum SRM sensitivity for PGF_{2α} and PGE₂. This type of curve is calculated by dividing the area of selected daughter ions, D_i , by the area

Figure 3. Pressure-resolved breakdown curve of the carboxylate anions of the MO-PFB-TMS derivatives of (a) $PGF_{2\alpha}$ and (b) PGE_2 .

of the incident parent ion, P_0 (measured in a daughter spectrum without collision gas). The reaction with the highest CAD efficiency should be selected to yield the highest sensitivity for SRM trace determination of prostaglandins. For example, in the case of $PGF_{2\alpha}$, choice of the 569⁻ → 299⁻ selected reaction would be the optimum at a collision pressure of 3.0 mTorr N_2 and collision energy of 30 eV. This reaction corresponds to the $[M-PFB]^- \rightarrow [(M-PFB) - 3(HOTMS)]^-$ for the derivatized carboxylate anion of $PGF_{2\alpha}$. For PGE_2 , the $[M-PFB]^- \rightarrow [(M-PFB) - 2(HOTMS) - CO_2 - HOCH_3]^-$ reaction is optimum at a pressure two times lower than for $PGF_{2\alpha}$. Even more notable is that the optimum CAD efficiency (D_i/P_0) for PGE_2 (10%) is five times higher than that for $PGF_{2\alpha}$ (2%).

The optimum collision gas pressure for both qualitative and quantitative (SRM) analysis of PGE_2 and $PGF_{2\alpha}$ is surprisingly different. Optimum pressures in both cases (Figures 3b and 4b) for PGE_2 are lower than the optima found for $PGF_{2\alpha}$ (Figures 3a and 4a). The dramatically lower CAD efficiency for the carboxylate anion of $PGF_{2\alpha}$ compared to that of PGE_2 clearly indicates its greater stability under CAD conditions.

In light of the dramatic differences observed in the CAD efficiencies of the carboxylate anions of the

fully derivatized $PGF_{2\alpha}$ and PGE_2 (differing structurally only in the presence of a carbonyl group at C-9 derivatized to a methoximine, in PGE_2 , rather than a hydroxyl group at the C-9 position, derivatized to a trimethylsilyl group, in $PGF_{2\alpha}$), two prostaglandins similar to these were studied. The PGD_2 and PGE_2 isomers vary only by the interchange of the hydroxyl and carbonyl groups on C-9 and C-11. $DHKF_{2\alpha}$ differs from $PGF_{2\alpha}$ by exchange of a carbonyl group (derivatized to a methoximine) for the hydroxyl group (derivatized to a trimethylsilyl) at C-15.

In Figure 5 curves for fragmentation, collection, and overall CAD efficiencies versus collision energy for the carboxylate anions of PGE_2 , $PGF_{2\alpha}$, PGD_2 and $DHKF_{2\alpha}$ are presented. These curves show the effects of varying the collision energy at two different collision gas pressures. In Figure 5a-c the collision pressure has been established at 1.2 mTorr N_2 , a value which is typically optimum for many MS/MS analyses. The fragmentation efficiency curve (Figure 5a) indicates the dramatic differences in stability of the carboxylate anions of the four PGs. At a collision energy of 30 eV the fragmentation efficiencies range from a typical 80% down to only 2%. The collision pressure must be increased to produce more efficient fragmentation. The collection efficiencies (Figure 5b) for the four PGs are

Figure 4. Overall CAD efficiency for SRM of the carboxylate anions of the MO-PFB-TMS derivatives of (a) $PGF_{2\alpha}$ and (b) PGE_2 .

Figure 5. CAD efficiency of the [MOX/TMS-PFB]⁻ carboxylate anions versus collision energy: (a) fragmentation at 1.2 mTorr N₂; (b) collection at 1.2 mTorr N₂; (c) overall CAD at 1.2 mTorr N₂; (d) fragmentation at 3.0 mTorr N₂; (e) collection at 3.0 mTorr N₂; (f) overall CAD at 3.0 mTorr N₂.

similar. The notable exception is PGD₂, which has an unusually high collection efficiency at collision energies of 10–25 eV. This explains the difference noted between the fragmentation efficiency of PGE₂ and PGD₂ compared to their overall CAD efficiency. Although there is no obvious explanation for the difference between the collection efficiencies for PGD₂ and PGE₂, it is reproducible ($\pm 10\%$) for triplicate runs.

Figure 5d–f shows the overall CAD, collection and fragmentation efficiencies at a collision gas pressure 2.5 times higher. The fragmentation efficiency (Figure 5d) as well as the overall CAD efficiency (Figure 5f)

curves demonstrate the differences in the stability of the carboxylate anions during the CAD process. At these high collision gas pressures, even at a collision energy as low as 4.0 eV, little or no parent ion is left for PGE₂ and PGD₂, yielding a fragmentation efficiency of nearly 100% (Figure 5d). The inefficient fragmentation (noted in Figure 5a) for PGF_{2 α} and DHKF_{2 α} is still observed. Note that at the higher collision gas pressure scattering losses become significant, especially at higher collision energies, yielding an optimum collision energy below the maximum available 30 eV.

The fragmentation efficiencies (E_F) for the carboxy-

Figure 6. CAD efficiency of the [MOX/TMS-PFB]⁻ carboxylate anions versus collision gas pressure at a collision energy of 30 eV: (a) fragmentation; (b) collection; (c) overall CAD.

late anions of the four PGs as a function of collision gas pressure at the maximum available collision energy (30 eV) are shown in Figure 6a. At low collision gas pressures PGE₂ has a fragmentation efficiency twenty times greater than that for PGF_{2α} and DHKF_{2α}. There are no dramatic differences in the collection efficiency (Figure 6b) of the four PGs, although at low collision gas pressures (<0.5 mTorr N₂), DHKF_{2α} has the highest E_C , but at high collision gas pressures the lowest E_C . Since the mass of the carboxylate anions are approximately the same, the increased loss of ions is probably not due to scattering but rather due to neutralization of the anion by electron detachment. The overall CAD

efficiencies (Figure 6c) for the carboxylate anions of the four PGs demonstrate the differences in their behavior; PGE₂ has a maximum E_{CAD} of 35%, which is two times greater than that for PGD₂, five times better than PGF_{2α}, and twenty times greater than DHKF_{2α}.

The efficiency curves for the four PGs demonstrate that differences do exist between F series PGs and PGE₂ and PGD₂. However, by examining Figure 6a-c, similarities can be noted for certain PGs. PGD₂ has a fragmentation, collection, and overall CAD efficiency behavior essentially the same as PGE₂. The behavior of DHKF_{2α} closely resembles that of PGF_{2α}. The notable difference is that DHKF_{2α} has an E_F and E_{CAD} even lower than that of PGF_{2α}. These results suggest that the presence of two trimethylsilyl groups on the cyclopentyl ring (positions C-9 and C-11) of PGF_{2α} and DHKF_{2α} add to the stability of the [M-PFB]⁻ carboxylate anion during CAD experiments.

Collision-activated Dissociation of the [M-PFB]⁻ Ions of Derivatized Prostaglandins

In order to test this hypothesis, the PFB ester derivatives (without methoxime or trimethylsilyl groups) of the PGs were examined. A conventional solids probe was used to introduce the derivatized PGs because, with only PFB derivatization, the PGs were no longer amenable to GC separation. The CAD efficiency data from this study are shown in Figure 7. The fragmentation behavior (Figure 7a) of the carboxylate anions of the PFB-only derivatives is similar to that observed for the fully derivatized PGs (Figure 6a). The PGF_{2α}-PFB and DHKF_{2α}-PFB derivatives fragment even less efficiently than the MO-PFB-TMS derivatives at collision pressures > 2.0 mTorr N₂. This noted difference in the fragmentation efficiency of the PFB derivatives is reflected in the extremely low overall CAD efficiency (Figure 7c) of PGF_{2α} and DHKF_{2α}.

The results from this study suggest that the presence or absence of trimethylsilyl and methoxime groups of the fully derivatized PGF_{2α}-PFB-TMS has little or no effect on the observed differences in the fragmentation behavior of PGE₂ and PGF_{2α}. It is hypothesized that the difference noted between the E_F of PGE₂ and PGF_{2α} is due only to the presence of two -OH (or -OTMS) groups on the cyclopentyl ring of the F-series prostaglandins. This is seen in Figure 8, which contains sketches of ball-and-stick models of PGE₂ and PGF_{2α}. Figure 8a shows one possible configuration of PGF_{2α} demonstrating how the carboxylate group can interact with the two -OH groups on C-9 and C-11. In contrast, the PGE₂ configuration (Figure 8b) shows that, with only one -OH group, the interaction with the carboxylate group may not be as strong. Thus, the stability of the carboxylate anions of the F series prostaglandins would be expected to be greater during CAD than the stability of the D and E series prostaglandins.

Figure 7. CAD efficiency of the $[M - PFB]^-$ carboxylate anions versus collision gas pressure at a collision energy of 30 eV: (a) fragmentation; (b) collection; (c) overall CAD.

Collision-activated Association of the $[M-H]^-$ Ions of Underivatized Prostaglandins

Underivatized prostaglandins were examined to look at the fragmentation and CAD efficiency of the $[M-H]^-$ carboxylate anions. These ions have nominally the same structures as the $[M-PFB]^-$ carboxylate anions formed by dissociative electron capture from the PFB-derivatized compounds. A conventional solids-probe was used for this study, as in the experiments done on the PFB-only derivatives ($[M-PFB]^-$). The $[M-H]^-$ ions of PGE₂ and PGD₂ could not be studied because of their low abundance (0.1% relative to $[M-H_2O]^-$)

in the EC-NCI mass spectrum; however, both PGF_{2α} and DHKF_{2α} produced an $[M-H]^-$ ion intense enough to permit CAD analysis. The E_F , E_C , and E_{CAD} for these nonderivatized $[M-H]^-$ ions are shown in Figure 9. The curve for E_C (Figure 9b) of the $[M-H]^-$ ion for DHKF_{2α} is similar to that found for the $[M-PFB]^-$ ion in Figure 7b; however, for PGF_{2α}, at lower collision pressures (<1.0 mTorr N₂), the E_C is three times higher than in the case of Figure 7b. Looking at Figure 9a and c, the fragmentation and CAD efficiencies are shown to be similar to those found in Figure 7a and c. The E_F and E_{CAD} for PGF_{2α} and DHKF_{2α} are two to four times greater for the $[M-H]^-$ ion than for the $[M-PFB]^-$ ion. This slight difference between the ions may be explained by the increased internal energy in the $[M-H]^-$ ion produced under EC-NCI compared to that of the $[M-PFB]^-$ ion produced by dissociative electron capture.

Conclusions

This study demonstrates the need for monitoring the CAD efficiency in the trace analysis of PGs. Optimization of both collision energy and collision gas pressure is essential in obtaining the optimum qualitative daughter spectrum. The CAD reaction with the highest CAD efficiency should be selected to maximize sensitivity for SRM determination of PGs. We have shown the drastic differences in the optimum collision gas pressures for SRM with MS/MS for the determination of PGE₂ and PGF_{2α}.

Figure 8. Sketch of ball-and-stick models of: (a) PGF_{2α} and (b) PGE₂. Solid balls represent oxygen atoms.

Figure 9. CAD efficiency of the $[M-H]^-$ carboxylate anions versus collision gas pressure at a collision energy of 30 eV: (a) fragmentation; (b) collection; (c) overall CAD.

The differences in fragmentation behavior of PGE_2 and $PGF_{2\alpha}$ were examined through the use of fragmentation, collection, and overall CAD efficiency studies. We have found that the CAD efficiency for the $[MOX/TMS-PFB]^-$, $[M-PFB]^-$ and $[M-H]^-$ carboxylate anions is significantly different for closely related PGs. Through the use of these curves, we have shown that F-series PGs fragment less efficiently than PGE_2 and PGD_2 . A theory has been proposed to explain these drastic differences seen in the fragmentation behavior of structurally similar carboxylate anions of PGs. It is believed that F-series PGs are more stable during CAD due to the interaction of the carboxylate group with the two -OH groups in the cyclopentyl ring.

References

1. Wu-Wang, C. Y.; Neu, J.; Gillespie, T. A.; Yost, R. A. *Seminars in Perinatology* **1986**, *10*, 255-269.
2. Blair, I. A.; Barrow, S. E.; Waddell, K. A.; Lewis, P. J.; Dollery, C. T. *Prostaglandins* **1982**, *23*, 579-589.
3. Barrow, S. E.; Waddell, K. A.; Ennis, M.; Dollery, C. T.; Blair, I. A. *J. Chromatogr.* **1982**, *239*, 71-80.
4. Waddell, K. A.; Blair, I. A.; Wellby, J. *Biomed. Mass Spectrom.* **1983**, *10*, 83-88.
5. Lawson, J. A.; Brash, A. R.; Doran, J.; Fitzgerald, G. A. *Anal. Biochem.* **1985**, *150*, 463-470.
6. Schweer, H.; Kammer, S.; Seyberth, H. W. *J. Chromatogr.* **1985**, *338*, 273-280.
7. Fischer, C.; Meese, C. O. *Biomed. Mass Spectrom.* **1985**, *12*, 399-404.
8. Meese, C. O.; Fischer, C.; Thalheimer, P.; Fürst, O. *Biomed. Mass Spectrom.* **1985**, *12*, 544-559.
9. Lawson, J. A.; Patrono, C.; Ciabattini, G.; Fitzgerald, G. A. *Anal. Biochem.* **1986**, *155*, 198-205.
10. Johnson, J. V.; Yost, R. A. *Anal. Chem.* **1985**, *57*, 758A-768A.
11. Kondrat, R. W.; Cooks, R. G. *Anal. Chem.* **1978**, *50*, 81A-92A.
12. Schweer, H.; Seyberth, H. W.; Schubert, R. *Biomed. Mass Spectrom.* **1986**, *13*, 611-619.
13. Schweer, H.; Seyberth, H. W.; Meese, C. O. *Biomed. Mass Spectrom.* **1988**, *15*, 129-138.
14. Schweer, H.; Seyberth, H. W.; Meese, C. O.; Fürst, O. *Biomed. Mass Spectrom.* **1988**, *15*, 143-152.
15. Hubbard, H. L.; Eller, T. D.; Mais, D. E.; Halushka, P. V.; Baker, R. H.; Blair, I. A.; Vrbanc, J. J.; Knapp, D. R. *Prostaglandins* **1987**, *33*, 149-159.
16. Yost, R. A.; Enke, C. G.; McGilvery, D. C.; Smith, D.; Morrison, J. D. *Int. J. Mass Spectrom. Ion Phys.* **1979**, *30*, 127-136.