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Analysis of some eicosanoids by continuous-flow fast atom bombardment mass spectrometry

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ABSTRACT

Continuous-flow fast atom bombardment has been used to analyze eicosanoids by selected-ion monitoring on a sector-field mass spectrometer operating in the negative-ion mode. The method has been optimized with respect to solvent composition and flow-rates. Detection limits were below 50 pg, and under optimal conditions a linear relationship between response and amount of substance was achieved. The method was succesfully applied to the analysis of two spiked urine samples.

INTRODUCTION

Mass spectrometric (MS) analysis of prostaglandins and other eicosanoids is commonly carried out by coupled gas chromatography–mass spectrometry (GC–MS) after derivatization [1–10]. The purpose of the present study was to investigate the possibility of analyzing these compounds by continous-flow fast atom bombardment (CF-FAB). The primary aim was to achieve a sensitivity that would make the method applicable to biological studies.

CF-FAB is most often used for analysis of positive ions [11–16], but some cases of analyzing negative ions are known [17]. With a sector instrument the stability of the accelerating voltage is a major problem under CF-FAB conditions. The flow of solvent and the ensuing high pressure in the source can cause discharges, the formation of which is promoted by ionizing collisions between Xe atoms and ions in the beam and the neutral molecules in the gas phase [18,19]. When the source is operated at high negative potentials, the secondary electrons produced by the Xe beam impact in the source are accelerated towards earth and become a further source of gas phase ionization leading to discharges.

To achieve maximum sensitivity selected-ion monitoring (SIM) of $[M-H]^-$ was employed, and we have optimized the method with respect to solvent composition and capillary diameter, we have also investigated the linearity of the response and performed recovery experiments using spiked urine samples.

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EXPERIMENTAL

Mass spectrometry

CF-FAB was carried out on a Kratos MS 50RF instrument (Kratos, Manchester, U.K.) with DS90 (Version 4.0) software and operating in the negative-ion mode. The slits were set to a resolution of 1000 (10% valley definition) and the accelerating voltage was set at 4 kV. For the SIM experiments the instrument was calibrated with a mixture of CsI and RbI in glycerol. The start mass was 310 and the cycle time 4 s. *m/z* 321.050 (protonated thioglycerol trimer) was used as lock-mass. The lock-mass dwell-time was set at 700–1000 ms. At shorter dwell-times the peaks would not be focused. The need for this long dwell-time probably reflects the fluctuating ion currents under CF-FAB compared to electron impact or chemical ionization.

The gun was an Ion-Tech saddle-field gun (Ion-Tech, Teddington, U.K.) operated with Xe at *ca.* 9 keV and a current of 1 mA indicated on the power supply. A laboratory-made probe, based on the standard Kratos design, was used for the continuous-flow inlet. The capillaries were made of deactivated fused silica as supplied by SGE (Melbourne, Australia). The O.D. was 0.3 mm and the I.D. 75 or 50 μm. The injector was a Rheodyne 7520 (Rheodyne, Cotati, CA, U.S.A.) with a loop volume of 0.5 μl. The capillary was fitted to the injector through a Vespel ferule. A Waters 6000 A pump (Millipore, Bedford, MA, U.S.A.) supplied the solvent to the injector through 1-m tubing (pressure equalizer) and a split. The flow-rate was usually 200 μl/min. The flow to the source (3–5 μl/min) was changed by adjusting the length of the capillary.

Materials

Prostaglandins (PG) and tromboxane B_2 (TXB_2) were supplied by Cayman Chemicals. ^{14}C-Labelled $PGF_{2\alpha}$ was from Amersham. Stock solutions were kept at −18°C and solvents for CF-FAB were thoroughly filtered before use.

Recovery experiments

A 1-μg amount of $PGF_{2\alpha}$ was added to 10 ml of urine. This relatively large amount of prostaglandin means that any natural background level can be ignored. After addition of the radioactively labelled $PGF_{2\alpha}$ the urine was treated as follows, according to the procedure described by Nigam [20]. The urine was adjusted to pH 3 with formic acid and applied onto a Sep-Pak C_{18} reversed-phase column (Waters), which was subsequently washed with 20 ml of 15% aquous ethanol, 20 ml of water and 20 ml of petroleum ether. The prostaglandins were eluted with 10 ml of freshly distilled methyl formate. After evaporation of the organic phase under argon, the residue was redissolved in 100 μl of mobile phase (water–acetonitrile–acetic acid, 67:33:0.1). High-performance liquid chromatography (HPLC) was carried out on a C_{18} reversed-phase column (250 mm × 4.6 mm I.D., Nucleosil 5 μm from Waters) under isocratic conditions. Fractions of 1

ml were collected and the fraction(s) with ^{14}C radioactivity (retention time *ca.* 14 min) were pooled and freeze-dried, and the expected amount of $PGF_{2\alpha}$ was calculated on the basis of the radioactivity recovered. For the CF-FAB analysis the pooled fractions were redissolved in an aqueous solution of the standard, 2,3-dinor-6-keto-$PGF_{1\alpha}$ (2 ng/μl). In experiment A, 200 μl were used and in experiment B 300 μl. Aliquots (0.5 μl) of the solutions were subsequently injected into the mass spectrometer and the relative height of the signal at *m/z* 353.233 ($PGF_{2\alpha}$) and *m/z* 341.196 (standard) was determined from the first six injections. From a standard curve, one for each of the experiments A and B, the amount of $PGF_{2\alpha}$ in 0.5 μl of the purified samples could be calculated and compared to the results based on the recovery of the radioactive label. Injections of freeze-dried and redissolved HPLC fractions without radioactivity showed only response from the standard.

RESULTS AND DISCUSSION

All results presented here have been obtained with thioglycerol as the non-volatile component of the solvent. The advantage of thioglycerol is illustrated in Table I. In this example TXB_2 occurs at the same nominal mass as an isotope peak from a glycerol cluster ion, which means that the background signal becomes very intense. With thioglycerol there is no cluster ion at this mass but only the ubiquitous chemical background. With thioglycerol, however, the chemical background, owing to the mass defect of sulphur, occurs at a lower exact mass, which further contributes to a weaker background signal. The background at *m/z* 369 from thioglycerol is assumed to arise from the loss of $C_2H_6O_2$ from the protonated trimer at *m/z* 427. However, the lower exact mass of the chemical background when thioglycerol is used instead of glycerol is a general phenomenon. Hence thioglycerol will produce a weaker background signal than glycerol at all the exact masses for $[M-H]^-$ ions from compounds that consist of only the elements C, H and O, such as the eicosanoids studied in this work.

TABLE I

ACCURATE MASSES OF $[M-H]^-$ FROM TXB_2 AND MATRIX IONS FROM GLYCEROL AND THIOGLYCEROL

Compound	Formula	*m/z*
$TXB_2([M-H]^-)$	$^{12}C_{20}{}^{1}H_{33}{}^{16}O_6$	369.22770
$[(Glyc)_3glyc\text{-}H]^-M+2$ isotope peak	$^{13}C_2{}^{12}C_{10}{}^{1}H_{31}{}^{16}O_{12}$	369.18824
Thioglycerol cluster fragment	$^{12}C_{10}{}^{1}H_{15}{}^{16}O_6{}^{32}S_4$	369.05339

With thioglycerol as the non-volatile component, it was difficult to obtain stable conditions on the probe with water as the volatile component. In order to obtain stable conditions, the volatile and non-volatile components have to disappear at the same relative rates as they are supplied. Since thioglycerol is considerably more volatile than glycerol, we decided to use methanol as the volatile component. This had the additional advantage that the pressure in the source did not become quite as high as with water, thus helping to maintain a stable accelerating voltage.

Fig. 1 shows a dose–response curve for arachidonic acid. The selected ion trace for the lowest amount is shown in Fig. 2. These results were obtained with a 75-μm capillary and a flow-rate of *ca.* 5.8 μl/min. The variations in the baseline indicate that the conditions on the probe were unstable. At flow-rates above *ca.* 4 μl/min heating of the probe is needed, but because of poor thermal contact between the source block which is heated in the standard design and the probe which is not heated, it was difficult to obtain consistent results at this flow-rate. Without heating from the source block, the probe is only heated by radiation from the gun. On our instrument this gives a stable temperature of *ca.* 40°C.

If the flow-rate was decreased to 3μl/min by using a longer capillary of the same I.D., the peaks broadened and the sample was eluted over *ca.* 3 min. Changing to a capillary with a smaller I.D. (50 μm) gave stable conditions on the target and resulted in narrow peaks (Fig. 3).

Fig. 4 shows the response for repeated injections of PGE_2 and $PGF_{2\alpha}$ (750 pg of each). It appears that $PGF_{2\alpha}$ gives a much stronger signal than PGE_2. The two

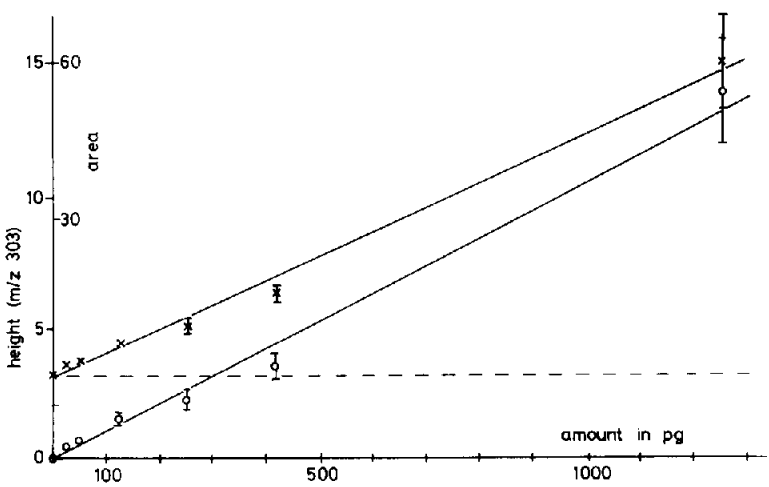

Fig. 1. Response (×, area; ○, height) at *m/z* 303 as a function of injected amount of arachidonic acid. Each point is the average of five or six injections. The error bars show the standard deviation and the lines are based on a linear regression: *r* (height) = 0.9770; *r* (area) = 0.9977. Capillary, 75 μm; solvent, thioglycerol–methanol (1:9); flow-rate, 5.8 μl/min.

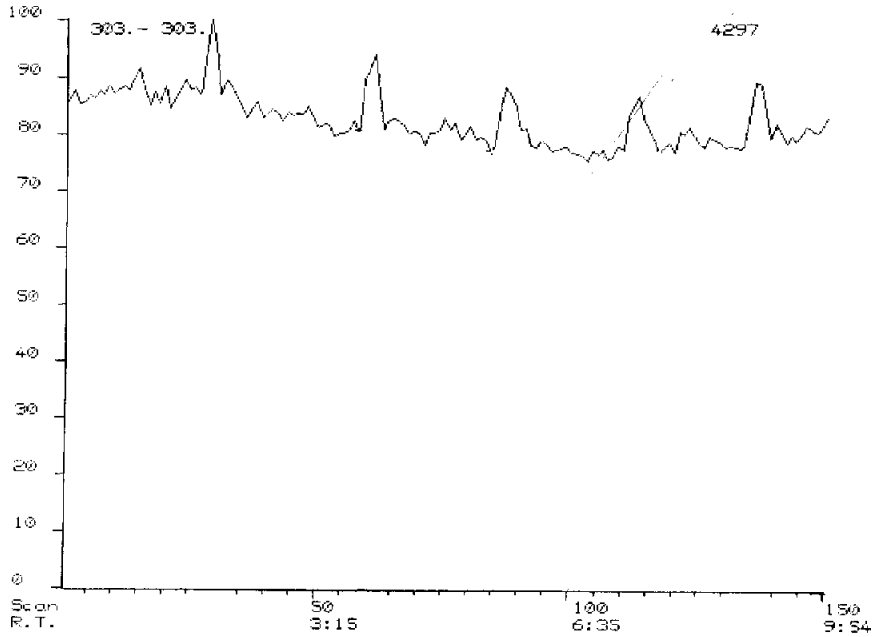

Fig. 2. Selected-ion trace of m/z 303 for six injections of 25 pg arachidonic acid. Conditions as for Fig. 1.

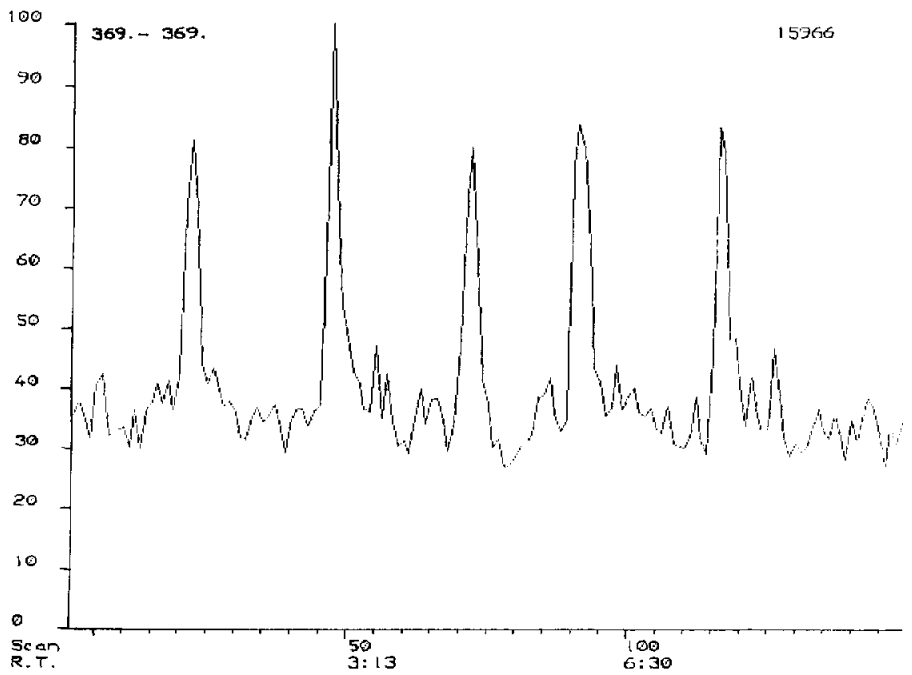

Fig. 3. Selected-ion trace of m/z 369 for five injections of 30 pg TXB_2. Capillary, 50 μm; solvent, thioglycerol–methanol (1:19); flow-rate, 3.5 μl/min.

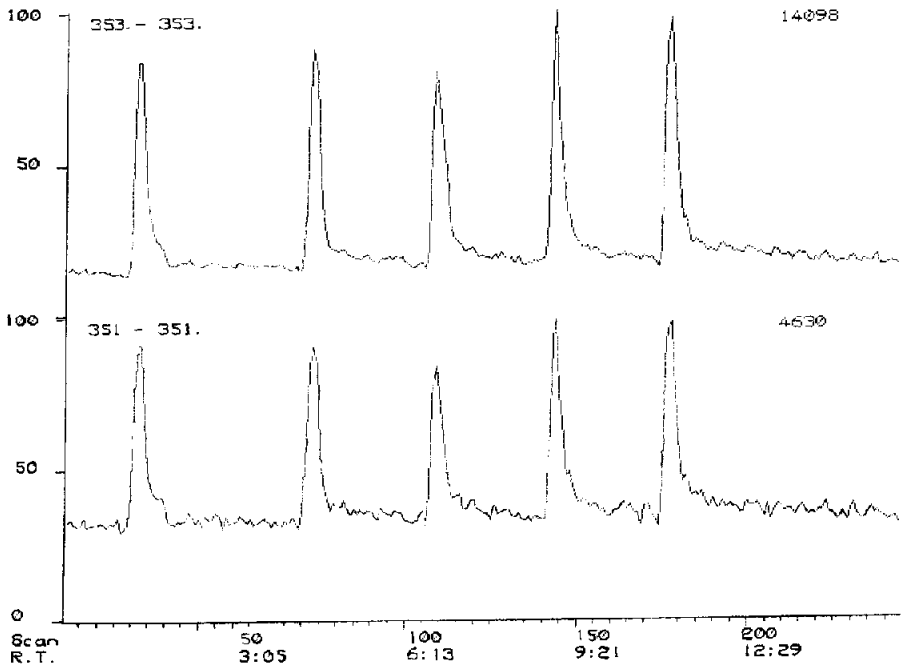

Fig. 4. Selected-ion trace of m/z 351 and m/z 353 for a mixture of 750 pg PGE_2 (m/z 351) and 750 pg $PGF_{2\alpha}$ (m/z 353). Capillary, 50 μm; solvent, thioglycerol–methanol (1:19); flow-rate, 3 μl/min.

compounds are identical except for a hydroxyl group in $PGF_{2\alpha}$, which in PGE_2 is oxidized to a ketone functionality. The linearity of the response is good for both compounds and independent of whether peak heights or areas are used. When mixtures (350, 750, 1750 and 3500 pg) of the two compounds were injected, linear dose–response curves were observed with $r \geqq 0.9975$. That linear dose–response curves can be obtained over such a wide range for a mixture of two compounds with very different ionization efficiencies is important when quantitative analysis with internal standards is considered.

TABLE II

RESULTS FROM TWO RECOVERY EXPERIMENTS OF $PGF_{2\alpha}$ FROM SPIKED URINE SAMPLES

Experiment	Amount $PGF_{2\alpha}$ in a 0.5-μl aliquot of purified sample (pg)	
	CF-FAB	^{14}C Recovery
A	305 ± 58	305
B	250 ± 19	265

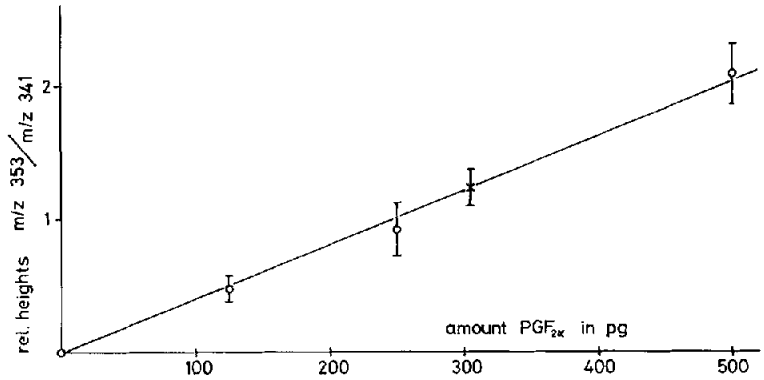

Fig. 5. Standard curve for $PGF_{2\alpha}$ (*m/z* 353). The standard was 2,3-dinor-6-keto-$PGF_{1\alpha}$ (*m/z* 341) with 1 ng in each injection. Each point is the average of six injections. The relative heights in the selected-ion trace were used. The error bars show the standard deviation and the line is based on a linear regression: r = 0.9954. The curve was used in recovery experiment A and the result for the purified urine sample is indicated by ×. Capillary, 50 μm; solvent, thioglycerol–methanol (1:19); flow-rate, 2.6 μl/min.

The method was tested by two recovery experiments of urine samples spiked with $PGF_{2\alpha}$. ^{14}C-Labelled $PGF_{2\alpha}$ was added to determine the recovery and calculate the expected amount of $PGF_{2\alpha}$ in the purified sample. The urine was purified according to the procedure described by Nigam [20]. The results of the two experiments are shown in Table II. The expected amount of $PGF_{2\alpha}$ based on the amount of radioactivity falls within the standard deviation of the value measured by CF-FAB. This measurement was performed with an internal standard added to the purified sample. The standard curve used in the first experiment is shown in Fig. 5.

CONCLUSION

The results presented here demonstrate the potential of CF-FAB for analysis of underivatized eicosanoids. More work is needed to test the precision of the method in quantitative analysis of biological samples. In terms of sensitivity the method compares favourably with thermospray ionization [21,22] and electron-impact ionization of derivatized samples [1–4,23], but it is not as sensitive as negative-ion chemical ionization of derivatized samples [2,5–10]. The overall sensitivity may increase by direct coupling of a chromatograph to the CF-FAB inlet. The change from glycerol to thioglycerol contributed greatly to the low detection limits and may well be an advantage when analysing other classes of compounds.

The weakest point in the experimental set-up we have used is probably the fitting of the capillary to the injector, where it was difficult to get a tight fit and low dead-volume. The problem of accelerating voltage instability was largely solved by using low flow-rates, but it always needed attention.

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