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DETERMINATION OF ACETYLTRIMOPROSTIL AND ITS METABOLITE TRIMOPROSTIL IN HUMAN OR DOG PLASMA BY GAS CHROMATOGRAPHY—NEGATIVE-ION CHEMICAL IONIZATION MASS SPECTROMETRY

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SUMMARY

A sensitive and specific procedure is described for the determination of the antisecretory prostaglandin acetyltrimoprostil and its metabolite trimoprostil in human or dog plasma using gas chromatography—negative-ion chemical ionization mass spectrometry (GC—NICI-MS). Trideuterated analogues of both compounds are added to plasma as the internal standards. The plasma is extracted at pH 7.3 with benzene—dichloromethane (9:1), and the residue of the organic extract is reacted at room temperature with pentafluorobenzyl bromide in the presence of 18-crown-6-ether and potassium acetate. The derivatives are reconstituted in heptane, and appropriate aliquots are analyzed by GC—NICI-MS with selected-ion monitoring of the intense $(M — C_6F_5CH_2)^-$ fragment ions of acetyltrimoprostil (m/z 419), trimoprostil (m/z 377), and their respective trideuterated analogues (m/z 422 and m/z 380, respectively). Quantitation of an experimental plasma sample is based on a comparison of the m/z 419 versus m/z 422 and m/z 377 versus m/z 380 ion ratios in each sample to that obtained from the analysis of drug-free plasma fortified with various amounts of both protio compounds, and a fixed amount of each trideuterated internal standard. The limit of quantitation of the assay for human plasma is 0.2 ng ml^{-1} with mean relative standard deviations at this concentration of 15.5% and 9.7% for acetyltrimoprostil and trimoprostil, respectively.

INTRODUCTION

Acetyltrimoprostil (I) is currently being studied for use as a long-acting, orally active inhibitor of gastric acid secretion [1]. Trimoprostil (II), a known metabolite of I, has well established antisecretory activity in dog [2] and in man [3, 4].

The published [5] gas chromatographic—negative-ion chemical ionization
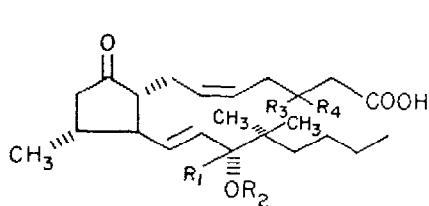
Compound	R_1	R_2	R_3	R_4
I	H	$COCH_3$	H	H
$[^2H_3]I$	^2H	$COCH_3$	^2H	^2H
II	H	H	H	H
$[^2H_3]II$	^2H	H	^2H	^2H

Fig. 1. Chemical structures of acetyltrimoprostil (I), trimoprostil (II) and their trideuterated analogues ($[^2H_3]I$ and $[^2H_3]II$, respectively).

mass spectrometric (GC—NICI-MS) assay for II could not be modified to determine I in human plasma because of interferences in the selected-ion current profiles from endogenous compounds. This paper reports a GC—NICI-MS procedure to determine both I and II in the same sample. This assay as the previously reported assay [5] also features the use of both the pentafluorobenzyl (PFB) derivatives of I and II and trideuterated analogues of each compound ($^2H_3[I]$ and $[^2H_3]II$, respectively). Unlike the previously reported assay [5], this assay features a packed GC column instead of a support-coated open tubular capillary column, determines II without silylating the hydroxy group, extracts I and II at a neutral rather than an acidic pH, and derivatizes the analytes under conditions where endogenous phenols would be expected to be unreactive. The assay was used to determine I and II in human and dog plasma following oral administration of I.

EXPERIMENTAL

Gas chromatography

A Finnigan (Sunnyvale, CA, U.S.A.) Model 9500 gas chromatograph was equipped with a 1.5 m × 2 mm I.D. U-shaped borosilicate glass column packed with 3% SP-2250 on 80—100 mesh Supelcoport from Supelco (Bellefonte, PA, U.S.A.). The column was conditioned at 320°C overnight with nitrogen as the GC carrier gas. For the assay, methane (2.4 kg cm^{-2}) was used as the GC carrier gas. The injector, column, interface oven and transfer line were operated at 320°C, 315°C, 240°C and 240°C, respectively. The retention times of derivatized I and II under these conditions were 165 and 175 sec, respectively. Prior to use, the GC column was conditioned with several injections of both Silyl-8 (Pierce, Rockford, IL, U.S.A.) and the reconstituted residue from an ethyl acetate extract of drug-free plasma.

Mass spectrometer

The ion source parameters of a Finnigan Model 3200 quadrupole mass spectrometer were set to give the maximum response consistent with reasonable ion peak shape and unit resolution. The modifications to the instrument to permit the detection of negative ions have been described [6]. The continuous-dynode electron multiplier was set to —2.0 kV, and the conversion dynode was set to +2.9 kV. Methane (Liquid Carbonic, 99%, Harrison, NJ, U.S.A.) at an ion source pressure of 68 Pa was used as NICI reagent gas.

Peak monitor

A Finnigan Promim® with a Rikadenki four-channel recorder was used to set the mass spectrometer to monitor m/z 419 (I), m/z 422 ($[^2H_3]$I), m/z 377 (II), and m/z 380 ($^2H_3$[II]). All channels were operated at a gain of 10^{-8} A/V, 100-msec dwell time and a filter setting of 0.5 Hz.

Glassware

Culture tubes (Pyrex 9825) of 16 ml provided with Teflon®-lined screw caps were used for plasma extraction. Conical centrifuge tubes (Pyrex 8061) of 5 ml were used for the evaporation of the organic extract. All the tubes were purchased from SGA Scientific (Bloomfield, NJ, U.S.A.). Prior to use, the glassware was treated with Siliclad® (Clay Adams, Parsippany, NJ, U.S.A.) and rinsed with methanol and dichloromethane.

Solvent evaporator

Solvents were removed at 45°C using a nitrogen evaporator (N-Evap®, Organomation Assoc., South Berlin, MA, U.S.A.).

Shaker

Extractions were carried out by shaking (60 strokes min^{-1}) on a variable-speed reciprocating shaker (Eberbach, Ann Arbor, MI, U.S.A.).

Centrifuge

Centrifugation was carried out on a Damon/IEC Model CRU-5000 refrigerated centrifuge (Needham Heights, MA, U.S.A.) operated at 1600 g and 10°C.

Chemicals

Methanol, benzene, methylene chloride and heptane were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). PFB bromide (PFB-Br) and 18-crown-6-ether were obtained from Aldrich (Milwaukee, WI, U.S.A.). Potassium acetate, potassium phosphate (KH_2PO_4) and sodium hydroxide were purchased from Mallinckrodt (St. Louis, MO, U.S.A.), Baker (Phillipsburg, NJ, U.S.A.), and Fisher Scientific (Pittsburgh, PA, U.S.A.), respectively. Compounds I, II, $[^2H_3]$I and $[^2H_3]$II were obtained from Dr. G. Holland, Chemical Research Department, Hoffmann-La Roche (Nutley, NJ, U.S.A.).

Solutions

Separate stock solutions containing 1.00 mg ml^{-1} of compounds I, $[^2H_3]$I, [II] or $[^2H_3]$II were prepared in methanol. An aliquot of each stock solution was diluted with methanol to give a working solution containing 400 ng ml^{-1}. Spiking solutions of I and II were prepared by diluting the appropriate working solutions with methanol to give solutions containing either 0.2, 0.5, 4, 10, 20 or 50 ng of I and II per 50 μl. Spiking solutions containing either 2 ng or 5 ng of both trideuterated internal standards per 50 μl were prepared in methanol from their respective working solutions.

1 M Phosphate buffer (pH 7.3). Monobasic potassium phosphate (136 g)

was disolved in 1 l of distilled water and the solution was adjusted to pH 7.3 with 5 M sodium hydroxide.

Benzene—methylene chloride (9:1) solution. Methylene chloride (100 ml) was diluted with 900 ml of benzene.

0.5% PFB-Br solution. A 50-μl volume of neat PFB-Br was added to 10 ml of benzene containing 1.5 mg ml^{-1} of 18-crown-6-ether, and the mixture was rapidly mixed by vortexing.

Clinical samples

Four normal healthy subjects (mean weight ± S.D. = 75 ± 15 kg) were fasted overnight prior to receiving a single oral dose of 9 mg of I. Blood samples (8 ml) were drawn into a glass vacutainer (Becton-Dickinson No. 6527) at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 8, 12, 16 and 24 h post-dose. Plasma, isolated after centrifugation, was stored at $-20°$C.

Dog samples

Five male beagle dogs, with an average body weight of 10 kg, received a single oral dose of 0.16 mg kg^{-1} I. Blood was drawn at 0, 0.5, 1, 2, 4, 6, and 24 h post-dose. The plasma, isolated after centrifugation, was stored at $-20°$C.

Calibration curve samples

Human plasma. Drug-free human plasma (1 ml) was added to culture tubes containing 20, 10, 4, 0.5, 0.2 or 0 ng of compound I and II, and 2 ng of each trideuterated internal standard. Samples were prepared in duplicate.

Dog plasma. Drug-free dog plasma (1 ml) was added to culture tubes containing 50, 20, 10, 4, 0.5 or 0 ng of compound I and II, and 5 ng of each internal standard. Samples were prepared in duplicate.

Experimental samples

Aliquots (1 ml) of experimental plasma were added to culture tubes containing either 2 ng (human plasma) or 5 ng (dog plasma) of each trideuterated analogue.

Extraction

To each plasma sample was added 1 ml of 1.0 M phosphate buffer, pH 7.3. The mixture was rapidly mixed by vortexing, and then was extracted for 20 min with 6 ml of benzene—methylene chloride (9:1). The mixture was centrifuged for 10 min, 5 ml of each extract was transferred to a 5-ml centrifuge tube, and the organic solvent was evaporated to dryness at 45°C under a gentle stream of nitrogen.

Derivatization

The residue was dissolved in 60 μl of 0.5% PFB-Br followed by a few crystals of potassium acetate. The tubes were allowed to stand at room temperature for 60 min, before the benzene and excess PFB-Br were evaporated under nitrogen at room temperature. The residues were dissolved in 50 μl of heptane.

GC—NICI-MS analysis

Aliquots (2—4 μl) from each derivatized extract were injected onto the GC

column. Approximately 30 sec after an injection, the GC divert valve was closed, and 15 sec later the electron filament was turned on.

Calculations

The peak heights in the selected-ion current profile were measured with a ruler, and the ion ratios for m/z 419 versus m/z 422 and m/z 377 versus 380 were calculated. The ion ratio versus concentration data for both I and II were analyzed by weighted $(1/y)$ linear regression. Concentrations of both I and [II] in the experimental samples were calculated using the equation: x (ng) $= (R - b)/m$, where R is the experimental m/z ion ratio, and b (intercept) and m (slope) were constants generated by the linear regression analysis of the calibration curve data.

RESULTS AND DISCUSSION

The NICI mass spectra of the PFB derivative of I, $[^2H_3]I$, II and $[^2H_3]II$ show no molecular ions. Cleavage of the PFB—oxygen bond of the ester function produces an intense fragment ion at m/z 419 (approximately 44% of the base peak ion at m/z 359) in the mass spectrum of I, and at m/z 377 (base peak) in the mass spectrum of II. An additional fragment ion at m/z 359 in the
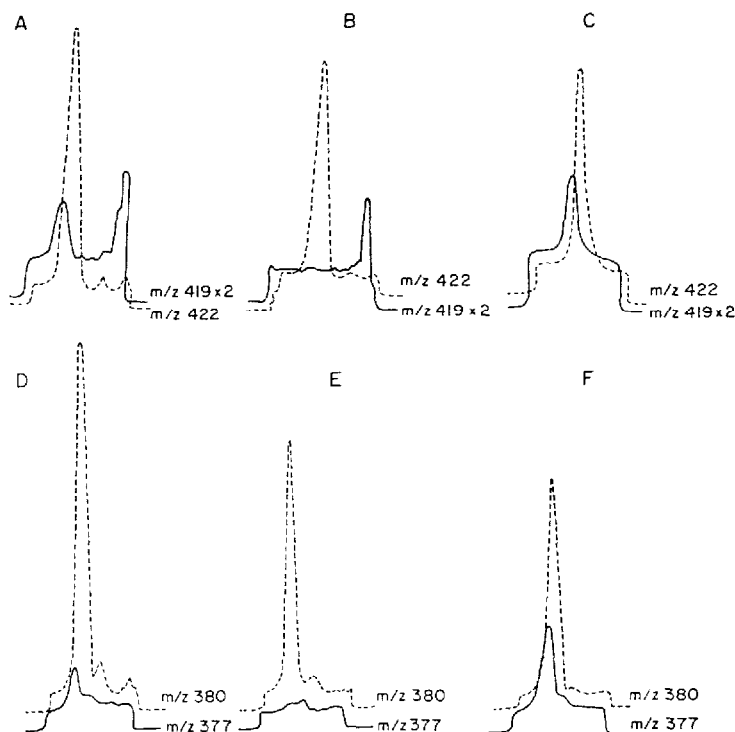

Fig. 2. Selected-ion current profiles from the analysis of either 1 ml of plasma from a dog at either 6 h (C and F) or 0 h (B and E) following an oral dose of 0.16 mg kg^{-1} I, or 1 ml of drug-free plasma fortified with 0.5 ng of both I and II (A and D). All samples were fortified with 5 ng of each trideuterated standard. Concentrations of I and II in the 6-h sample were 1.1 and 0.97 ng ml^{-1}, respectively.

TABLE I

INTER-ASSAY STATISTICS FOR THE ANALYSIS OF I AND II IN HUMAN PLASMA FROM A CONSIDERATION OF THE CALIBRATION CURVE DATA

$n = 14$ in all cases.

Amount added (ng/ml)	Compound I			Compound II		
	Found (ng/ml)	± S.D.	R.S.D.* (%)	Found (ng/ml)	± S.D.	R.S.D. (%)
0.2	0.18	0.03	15.50	0.18	0.02	9.68
0.5	0.48	0.03	6.35	0.51	0.02	4.81
4.0	4.24	0.17	3.91	4.29	0.33	7.71
10.0	10.41	0.26	2.53	10.42	0.35	3.39
20.0	19.44	0.38	1.95	19.39	0.44	2.27
		Mean =	6.05		Mean =	5.56

*R.S.D. = relative standard deviation.

TABLE II

INTRA-ASSAY STATISTICS FOR THE ANALYSIS OF I AND II IN HUMAN PLASMA FROM A CONSIDERATION OF THE CALIBRATION CURVE DATA

Values are the ratios obtained by dividing the first ion ratio of the duplicate pair by the second ion ratio in the pair. In all cases $n = 14$.

Calibration curve	0.2 ng/ml	0.5 ng/ml	4.0 ng/ml	10.0 ng/ml	20.0 ng/ml	
Compound I						
1	1.00	0.97	1.03	1.92	1.02	
2	0.94	0.97	1.02	1.01	1.01	
3	0.92	1.00	1.04	1.04	1.01	
4	1.09	1.03	1.05	0.97	1.03	
5	0.75	1.00	0.96	1.02	0.98	
6	0.80	0.93	0.96	1.03	0.98	
7	1.0	1.04	0.94	1.03	1.04	
Mean	0.93	0.99	1.00	1.00	1.02	
S.D.	0.12	0.04	0.05	0.04	0.03	
R.S.D.* (%)	13	4	5	4	3	Mean = 5.8
Compound II						
1	1.20	1.00	0.90	1.10	1.02	
2	1.00	1.10	1.05	0.96	1.05	
3	0.93	1.00	1.00	0.93	1.02	
4	1.00	0.91	0.89	0.95	1.06	
5	0.78	0.91	0.92	1.03	1.03	
6	1.00	1.00	1.04	1.02	1.01	
7	1.00	0.90	0.95	1.01	1.00	
Mean	0.98	0.97	0.96	1.00	1.03	
S.D.	0.12	0.07	0.07	0.06	0.02	
R.S.D. (%)	13	7.4	7.3	6	2	Mean = 7.1

*R.S.D. = relative standard deviation.

spectra of both I and II corresponds to the loss of the elements of acetic acid and water from the $[M — C_6F_5CH_2]^-$ ions of I and II, respectively. The spectra of the trideuterated analogues used in the assay as internal standards are identical to those of the protio compounds, except that the masses are shifted by the appropriate number of daltons.

Fig. 2 shows typical selected-ion current profiles from the analysis of either 1 ml of plasma from a dog at either 6 h (C and F) or 0 h (B and E) following an oral dose of 0.16 mg kg^{-1} I, or 1 ml of drug-free plasma fortified with 0.5 ng of I and II (A and D). All samples were fortified with 5 ng each of $[^2H_3]$I and $[^2H_3]$II. Similar profiles were obtained from the analysis of plasma samples from humans who had received a single oral dose of 9 mg of I. The limit of quantitation of the assay for human plasma samples was 0.2 ng ml^{-1} using 2 ng of each deuterated· internal standard. For dog plasma samples the limit of quantitation was 0.5 ng ml^{-1} using 5 ng of each deuterated internal standard. An injection of approximately 4 pg of either derivatized I or II gave a single-ion current profile with a signal-to-noise ratio of approximately 5:1.

Much simpler selected-ion current profiles were observed if the silylation reaction used in the previous assay [5] for II was omitted. In this regard, it was observed that prostaglandins and related analogues not having a gem-di-methyl group adjacent to the hydroxy groups could not be analyzed by GC without first protecting the hydroxy groups. Other modifications increasing assay specificity were the use of the less basic potassium acetate, instead of diisopropylethylamine, to catalyze the esterification reaction, and the extraction of the plasma at pH 7.3 instead of pH 3.0. The former modification
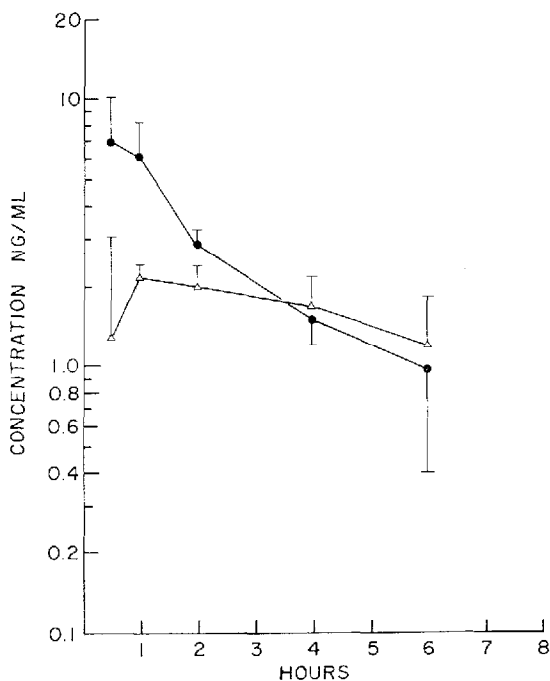

Fig. 3. Mean plasma concentrations (± S.D.) of I (•) and II (△) in five male dogs following an oral dose of 0.16 mg kg^{-1} I.
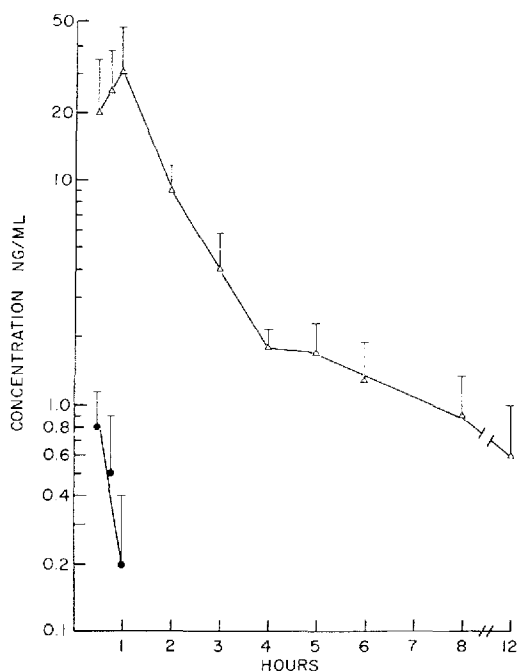
Fig. 4. Mean plasma concentrations (± S.D.) of I (●) and II (△) in four humans (mean weight ± S.D. = 75 ± 15 kg) who received a 9-mg dose of I.

prevents derivatization of endogeneous phenols, while the latter modification decreases the extraction of many endogeneous organic acids.

Inter- and intra-assay precision from a consideration of calibration curve data prepared from human plasma are given in Tables I and II, respectively. The overall inter-assay precision is approximately 6%, while the precision at the limit of quantitation (0.2 ng ml^{-1}) is 15%. Results for dog plasma are similar except the limit of quantitation is 0.5 ng ml^{-1}.

Mean concentrations of I and II in human and dog plasma following oral administration of either 0.16 mg kg^{-1} I to dogs or approximately 0.12 mg kg^{-1} I to man are given in Figs. 3 and 4, respectively. In man, the dose results in relatively high concentrations of II with respect to that of I (which could only be determined for 1 h post-dose). In the dog, the concentrations of I and II are nearly equal and could be determined for up to 6 h postdose.

REFERENCES

1 T. Gaginella, R. Bertko, G. Holland, P. Rosen, H. Gallo-Torres and A.C. Sullivan, Gastro-enterology, 83 (1983) 1161.
2 D.E. Wilson, W. Chang, J. Palsrud and G. Holland, Prostaglandins, 16 (1978) 121.
3 D.E. Wilson and S.L. Winter, Prostaglandins, 16 (1978) 127.
4 G. Friedman, Amer. J. Gastroenterol., 78 (1983) 387.
5 B.H. Min, J. Pao, W.A. Garland, J.A.F. de Silva and M. Parsonnet, J. Chromatogr., 183 (1980) 411.
6 W.A. Garland and B.H. Min, J. Chromatogr., 172 (1979) 279.