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ASSAY METHODS FOR 6-KETO-PROSTAGLANDIN $F_{1\alpha}$ IN HUMAN URINE

COMPARISON OF CHROMATOGRAPHIC TECHNIQUES WITH RADIOIMMUNOASSAY AND GAS CHROMATOGRAPHY—NEGATIVE-ION CHEMICAL-IONIZATION MASS SPECTROMETRY

ROBERT D. ZIPSER*

Department of Medicine, Harbor-UCLA Medical Center, 1000 West Carson Street, Torrance CA 90509 (U.S.A.)

AUBREY MORRISON

Departments of Medicine and Pharmacology Washington University School of Medicine, St. Louis, MO 63110 (U.S.A.)

and

GIACOMO LAFFI* and ROBERT DUKE

Department of Medicine, Harbor-UCLA Medical Center, 1000 West Carson Street, Torrance, CA 90509 (U.S.A.)

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SUMMARY

6-Keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) in human urine is considered to be a reflection of renal prostacyclin production. Because of the large amounts of unidentified eicosanoid metabolites in urine that may potentially bind to 6-keto-$PGF_{1\alpha}$ antisera, most radioimmunoassays include chromatographic purification of urine. A comparison of chromatographic techniques and of antisera to 6-keto-$PGF_{1\alpha}$ for the assay of human urine is described. Gas chromatography—negative-ion chemical-ionization mass spectrometry (GC—NICI-MS) was used as the reference method. Radioimmunoassays were performed with each of four

*Visiting scholar from Istituto di Clinica Medica IV, Universita degli Studi di Firenze, Florence, Italy.

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antisera combined with each of three chromatographic purification systems (silicic acid, Sephadex LH-20, reversed-phase high-performance liquid chromatography). There was marked variability in the results; however, there was at least one chromatographic method for each antiserum that yielded results comparable to GC—NICI-MS. Direct radioimmunoassay of urine without chromatography yielded markedly elevated and variable results for the four antisera. In contrast, the four antisera gave very similar results with direct assay of media from isolated perfused organs. Thus, for the radioimmunoassay of 6-keto-$PGF_{1\alpha}$ in human urine, each antiserum is sensitive to different contaminants in urine and must be individually matched to a chromatographic purification system.

INTRODUCTION

Prostacyclin (PGI_2) is a major cyclooxygenase product of arachidonic acid, with potent vasodilatory and platelet anti-aggregatory properties, that is produced in many biological tissues, including the kidney. PGI_2 is unstable in biological media, and a fraction is hydrolyzed to an inactive product, 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$). Urinary excretion of 6-keto-$PGF_{1\alpha}$ is generally assumed to reflect renal production of PGI_2, and a variety of radioimmunoassay (RIA) [1—7] and mass fragmentation assay [2, 8, 9] techniques have been reported for urinary 6-keto-$PGF_{1\alpha}$.

Urinary 6-keto-$PGF_{1\alpha}$ RIAs are particularly sensitive to unidentified cross-reacting compounds, and most of the reported techniques require substantial purification with extraction and chromatography of urine prior to assay or the conversion of 6-keto-$PGF_{1\alpha}$ to a derivative [1]. The chromatographic techniques include silicic acid [2], Sephadex LH-20 [3, 4], reversed-phase high-performance liquid chromatography (HPLC) [5], and sequential silicic acid and HPLC [6, 7]. It is not clear if one chromatographic system is more advantageous than the other systems in isolating 6-keto-$PGF_{1\alpha}$ from cross-reacting impurities; although some laboratories have documented better isolation with HPLC than with silicic acid chromatography for their antisera [6]. Our study was designed to systematically compare these chromatographic systems with different 6-keto-$PGF_{1\alpha}$ antisera to determine if there is one preferable chromatographic system for all of the antisera or if there are differing characteristics of the antisera that require different chromatographic systems for accurate assay of urinary 6-keto-$PGF_{1\alpha}$. The reference method for this study is combined capillary column gas chromatography—negative-ion chemical-ionization mass spectrometry (GC—NICI-MS).

EXPERIMENTAL

Biological samples

For comparison of assay methods, eight urine samples were collected from healthy adult volunteers, two men and two women. The four basal samples were obtained from 8-h overnight collections. Two of the four subjects received indomethacin 50 mg orally prior to the 8-h collection. Two of the four subjects received furosemide 60 mg intravenously prior to a 4-h collection. Urine was collected in glass bottles with PTFE tops, divided into aliquots for RIA and for MS analysis, and immediately frozen at −30° C.

Additional assay comparisons utilized nine samples of Krebs-Henseleit media from isolated perfused rabbit colons [10]. Some aliquots were obtained after bradykinin or angiotensin infusion, chosen to provide a range of 6-keto-$PGF_{1\alpha}$ concentrations from 780 to 18 400 pg/ml based on the initial assays.

Gas chromatography—mass spectrometry

GC—NICI-MS assays were performed on six of the samples (basal and furosemide) using a modification of the method of Barrow et al. [9]. In brief, 1- or 2-ml aliquots of urine are equilibrated with 10 ng of [3,3',4,4'-$^2H_4$]6-keto-$PGF_{1\alpha}$ (Merck-Frosst, Quebec, Canada), adjusted to pH 3.5 with hydrochloric acid, and extracted with 1 g C_{18} reversed-phase octadecylsilyl silica columns (Baker, Phillipsburg, NJ, U.S.A.) using sequential washing with distilled water, 15% ethanol, light petroleum and collecting with methyl formate [11].

The extract was dried and converted to the methoxime (MO) derivative by overnight incubation at room temperature with 50 μl methoxyamine hydrochloride in anhydrous pyridine (5 mg/ml). Pyridine was evaporated under nitrogen, and the sample was purified by thin-layer chromatography (TLC) using a solvent system of water-saturated ethyl acetate—2,2,4-trimetylpentane—acetic acid (110:50:20). The 6-keto-$PGF_{1\alpha}$ MO derivative zone was scraped from the TLC plate, eluted with methanol, and dried under nitrogen. The pentafluorobenzyl (PFB) ester was prepared by dissolving the extract in 30 μl of acetonitrile, adding 10 μl of PFB bromide in acetonitrile and 10 μl of N,N-diisopropylethylamine, and incubating at 40° C for 30 min. The samples were dried and converted to the trimethylsilyl (TMS) derivative by overnight incubation with 125 μl bis(trimethylsilyl)trifluoroacetamide (Pierce, Rockford, IL, U.S.A.). The samples were dried, and the MO-PFB-TMS derivatives were ready for assay.

GC—MS utilized a Hewlett-Packard 5985B machine in the NICI mode with a Grob injector. Chromatography was carried out on a wide-bore cross-linked OV-1 capillary column (25 m). Helium was the carrier gas, and the column temperature was programmed to increase from 85° C to 250° C at 30° C/min after injection. Methane was used as the ionization gas for NICI-MS. Selective-ion monitoring (SIM) was performed using fragment ions *m/z* 614 and 618 for ^1H and ^2H, respectively. The standard curve ranged from 2 to 200 pg based on peak area ratio of $^1H/^2H$. Intra-assay and inter-assay coefficients of variation are less than 10%.

Radioimmunoassay reagents

All solvents were of the highest quality, distilled in glass (Burdick & Jackson Labs., Muskegon, MI, U.S.A.). The [^3H]6-keto-$PGF_{1\alpha}$ (150 Ci/mmol) was purchased from New England Nuclear (Boston, MA, U.S.A.). Prostaglandin standards were purchased from Upjohn (Kalamazoo, MI, U.S.A.) or were a gift from Dr. J. Pike. Silicic acid (Unisil), 100—200 mesh, was purchased from Clarkson Chemical (Williamsport, PN, U.S.A.) and Sephadex LH-20 from Pharmacia (Uppsala, Sweden). 6-Keto-$PGF_{1\alpha}$ antiserum was obtained from four laboratories: antiserum 1 was raised at the University of Southern California (Los Angeles, CA, U.S.A.) as previously reported [3]; antiserum 2

was raised at Washington University (St. Louis, MO, U.S.A.); antiserum 3 was a gift of Dr. L. Levine of Brandeis University (Waltham, MA, U.S.A.); and antiserum 4 was a gift of Dr. D. Felson (Cornell University, New York, NY, U.S.A.) and Dr. T. Lysz (New Jersey Medical School, NJ, U.S.A.). Goat anti-rabbit antiserum was purchased from Antibodies (Davies, CA, U.S.A.).

Extraction and chromatography

About 1500 cpm of [^{3}H]6-keto-PGF$_{1\alpha}$ were added to 20-ml aliquots of urine, the samples acidified to pH 4.3 with hydrochloric acid, extracted with 35 ml of ethyl acetate, and dried under nitrogen. The extract was then subjected to chromatography as described below. The peak 6-keto-PGF$_{1\alpha}$ chromatography fraction was divided into five aliquots for recovery calculations and for simultaneous assay with the four antisera.

Silicic acid chromatography. Columns were prepared by slurrying 0.5 g of silicic acid in 4 ml of benzene—ethyl acetate (60:40). After application of the extract, the column was washed with 3 ml of benzene—ethyl acetate (60:40), and the 6-keto-PGF$_{1\alpha}$ fraction eluted with 5 ml of benzene—ethyl acetate—methanol (60:40:20). Recovery of [^{3}H]6-keto-PGF$_{1\alpha}$ averaged 67%.

Sephadex LH-20 chromatography. The extracts were applied to 30 × 0.5 cm columns of Sephadex LH-20 dissolved in dichloromethane—methanol (95:5) and chromatographed with the same solvent system. Effluents (1 ml) from the column were collected. 6-Keto-PGF$_{1\alpha}$ elutes at 11 ml. Recovery of [^{3}H]6-keto-PGF$_{1\alpha}$ averaged 42%. This method is identical to our method for prostaglandin E_2 (PGE$_2$) RIA [12] and differs from the original 6-keto-PGF$_{1\alpha}$ assay [3] that uses a 60 × 1.0 cm column with 10-ml collections (peak 6-keto-PGF$_{1\alpha}$ fraction is at about 110 ml).

Reversed-phase HPLC. A 10-μm fatty acid analysis reversed-phase column (Waters Assoc., Milford, MA, U.S.A.) was used with an isocratic solvent system containing acetonitrile—water—benzene—acetic acid (30.0:69.7:0.2:0.1) with a flow-rate of 2 ml/min. Fractions of 0.5 ml are collected. 6-Keto-PGF$_{1\alpha}$ elutes at 4 ml. Recovery of [^{3}H]6-keto-PGF$_{1\alpha}$ averages 62%. This system is a modification of the methods of Whorton et al. [13] and of Alam et al. [14] which use 23.0% acetonitrile. Our modification provides fractions of smaller volume with less water. A similar modification is used for the assay of urinary thromboxane B_2 (TxB$_2$) [15].

Straight-phase HPLC. A 10-μm μPorasil silicic acid column (Waters Assoc.) was used with a two-pump programmable solvent delivery system (Altex 110A, Cole Scientific, Calabasas, CA, U.S.A.). The extract was eluted with a linear gradient of chloroform to chloroform—methanol—acetic acid (89.1:8.1:2.0) over 10 min at a flow-rate of 1 ml/min followed by isocratic flow at 1 ml/min with collection of 0.5-ml fractions. 6-Keto-PGF$_{1\alpha}$ elutes at 20 ml, and final recovery of reversed-phase followed by straight-phase HPLC averaged 33%. Only antisera 1 and 2 were evaluated with the sequential reversed-phase and straight-phase HPLC method.

Radioimmunoassay procedure

All RIAs used a final volume of 250 μl with separation of bound from free with second antiserum technique as described for PGE$_2$ [12] and for TxB$_2$

[15]. As some chromatographic solvents affect the standard curve of some of the RIA systems, aliquots of eluting solutions were added to standards. Calculations were corrected for the [^{3}H]6-keto-PGF$_{1\alpha}$ counts used for recovery. The final titer of antisera was 1:1000 for antiserum 1, 1:1500 for antiserum 2, 1:6000 for antiserum 3, and 1:4000 for antiserum 4. Cross-reactivity testing for the four antisera is shown in Table I. The B_0 and 50% displacement values were very similar on repeated assays using a single RIA system (tested with antisera 1 and 2). These values were somewhat altered by different chromatographic system effluents. In general the B_0 (percentage of tracer bound to the antisera in the absence of standards) was about 50% displacement of tracer for antiserum 1, 60% for antiserum 2, 50% for antiserum 3, and 35% for antiserum 4 for the titers of antiserum dilution listed above. The 50% reduction of binding (assigning B_0 to 100%) corresponded to about 100 pg for each of the antisera. Non-specific binding was generally less than 5 pg for all antiserum chromatography systems. Radioactivity was quantitated with a Beckman LS 7800 scintillation counter (Fullerton, CA, U.S.A.) using Budget-solve scintillation cocktail (Research Products, Prospect, IL, U.S.A.). The method blank was determined by adding [^{3}H]6-keto-PGF$_{1\alpha}$ to buffer followed by extraction and chromatography in parallel with urine samples. Blank values were generally less than 10 pg for all Sephadex LH-20 and HPLC chromatographic systems, but ranged up to 40 pg for some of the silicic acid systems. Standard validation procedures of sample dilution (r = 0.98) and addition of known amounts of 6-keto-PGF$_{1\alpha}$ to urine samples (r = 0.98) for antiserum 1 with LH-20 chromatography (60 × 1 cm columns) were described previously [3]. Similar studies for antiserum 2 with reversed-phase HPLC revealed correlation of predicted and measured r = 0.86 and r = 0.97 for dilution and addition of standards, respectively.

A direct assay without extraction or chromatography was also performed with each of the antisera. For this method 50 μl of urine were added to a mixture of buffer, first antiserum and tracer and then combined with second antiserum. A similar procedure was used for assay of 50-μl aliquots of effluent from Krebs-Henseleit perfused rabbit colons (see Table VI). The Krebs-Henseleit medium was also added to the standards.

TABLE I

CROSS–REACTIVITY (%) COMPARISONS OF 6-KETO-PGF$_{1\alpha}$ ANTISERA

Anti-serum	6-Keto-PGF$_{1\alpha}$	PGE$_2$	PGF$_{2\alpha}$	TxB$_2$	PGD$_2$	Dinor-6K-PGF$_{1\alpha}$*	dH-dK-PGF$_{1\alpha}$**	dH-dK-Dinor-PGF$_{1\alpha}$***	dH-dK-Trinor-PGF$_{1\alpha}$§
1	100	<0.1	2.0	—	<0.1	35.0	0.5	0.4	<0.1
2	100	<0.1	2.0	<0.1	<0.1	3.3	0.8	0.5	<0.1
3	100	<0.1	3.6	<0.1	<0.1	9.5	0.8	0.3	<0.1
4	100	<0.1	2.0	<0.1	<0.1	8.5	0.7	0.2	<0.1

*2,3-Dinor-6-keto-PGF$_{1\alpha}$.
**13,14-Dihydro-6,15-diketo-PGF$_{1\alpha}$.
***13,14-Dihydro-6,15-diketo-2,3-dinor-PGF$_{1\alpha}$.
§19-Carboxy-13,14-dihydro-6,15-diketo-2,3,20-trinor-PGF$_{1\alpha}$.

TABLE II

OVERALL COMPARISON OF RIA TO GC—MS

Sample	Urinary 6-keto-$PGF_{1\alpha}$ (ng/h)		
	Chromatography		Direct
	GC—MS	RIA*	RIA**
Basal	8.9	9.6	70.5
Furosemide	36.7	31.9	106.0

*Overall mean of four antisera with each of three or four chromatographic systems.
**Mean results from RIA without extraction or chromatography with each of the four antisera.

TABLE III

COMPARISON OF ANTISERA AND CHROMATOGRAPHY SYSTEMS

Antiserum	Chromatography	6-Keto-$PGF_{1\alpha}$ (ng/h)		Correlation with GC—MS
		Basal	Furosemide	
1	Silicic acid	8.9	108.4	0.89
2	Silicic acid	9.0	79.7	0.80
3	Silicic acid	9.5	58.8	0.84
4	Silicic acid	5.3	61.3	0.66
1	Sephadex LH-20	8.3	18.8	0.59
2	Sephadex LH-20	12.0	10.7	0.23
3	Sephadex LH-20	2.3	—	0.16
4	Sephadex LH-20	5.3	4.8	0.12
1	HPLC*	10.1	10.2	−0.22
2	HPLC	21.7	47.7	0.81
3	HPLC	3.6	7.6	0.68
4	HPLC	18.3	24.2	0.01
1	Direct**	48.2	81.4	0.07
2	Direct	69.6	151.9	0.61
3	Direct	32.0	77.5	0.41
4	Direct	132.1	86.6	−0.45
—	GC—NICI-MS***	6.6	36.7	—

*Reversed-phase high-performance liquid chromatography.
**Without extraction or chromatography.
***Gas chromatography—negative-ion chemical-ionization mass spectrometry.

RESULTS

Table II lists the mean GC—NICI-MS results for basal and furosemide collection periods, the mean chromatography—RIA results as determined by combining measurements from all four antisera and all chromatographic systems, and the mean direct RIA results. Overall, the results of direct RIA were considerably higher than results from the other methods. Urinary 6-keto-$PGF_{1\alpha}$ measurements from basal urine samples averaged 8.9 ng/h by GC—NICI-MS, 9.6 ng/h by chromatography—RIA methods, and 70.5 by direct RIA.

TABLE IV

EFFECT OF SEQUENTIAL CHROMATOGRAPHY WITH REVERSED–PHASE, THEN STRAIGHT-PHASE HPLC ON URINARY 6-KETO-$PGF_{1\alpha}$ RIA (ng/h)

Samples	Antiserum 1		Antiserum 2	
	1st HPLC*	2nd HPLC**	1st HPLC	2nd HPLC
Basal	10.1	4.4	21.7	5.1
Indomethacin	10.8	2.7	19.0	4.5
Furosemide	10.2	14.4	47.7	7.9

*Reversed-phase HPLC.
**Reversed-phase HPLC followed by straight-phase HPLC.

TABLE V

COMPARISON OF RIA RESULTS OF THE MAJOR CHROMATOGRAPHY–RIA SYSTEMS USED WITH EACH ANTISERUM

Antiserum	Basal (ng/h)	Correlation with other antisera				Correlation with GC–MS
		1	2	3	4	
1 + Sephadex LH-20	8.3	—	0.92	0.70	0.74	0.59
2 + HPLC	21.7	0.92	—	0.82	0.69	0.81
3 + Silicic acid	9.5	0.70	0.82	—	0.98	0.84
4 + Silicic acid	5.3	0.74	0.69	0.98	—	0.66

TABLE VI

COMPARISON OF 6-KETO-$PGF_{1\alpha}$ ANTISERA FOR RIA OF KREBS MEDIUM FROM AN ISOLATED PERFUSED RABBIT COLON

Antiserum	6-Keto-$PGF_{1\alpha}$* (pg per 50 μl)	Correlation with other antisera			
		1	2	3	4
1	288 ± 96	—	0.89	0.83	0.98
2	248 ± 81	0.89	—	0.84	0.78
3	353 ± 106	0.83	0.84	—	0.79
4	243 ± 101	0.98	0.78	0.79	—

*Mean result of nine samples which encompass a wide range of 6-keto-$PGF_{1\alpha}$ concentrations.

Furosemide administration was associated with an increase in 6-keto-$PGF_{1\alpha}$ with GC–NICI-MS and with all types of RIA. A single oral dose of indomethacin resulted in suppression ranging up to about 40% with some of the chromatography–RIA systems.

Comparison of the four antisera with each of the chromatographic systems is shown in Table III. There is considerable variation in the correlation with GC–NICI-MS. Eight of the eleven chromatography–RIA techniques and three of the four direct assays showed increased 6-keto-$PGF_{1\alpha}$ excretion with furosemide.

The effect of sequential chromatography with reversed-phase HPLC followed by straight-phase HPLC on urinary 6-keto-$PGF_{1\alpha}$ measurements is shown in Table IV. The addition of the second HPLC system tended to reduce the calculated results. Although these basal values more closely resembled those of GC—NICI-MS, post-furosemide results were considerably lower than those of GC—NICI-MS.

A comparison of four chromatography—RIA systems is shown in Table V. These are the most popular chromatographic systems used for each of the four antisera as determined from publications and from discussions with the laboratories that use these antisera for urinary assays. Comparison of these RIA methods suggests very good correlations compared with each other and with GC—NICI-MS. All of these methods appear to be reasonable techniques for assay of urinary 6-keto-$PGF_{1\alpha}$.

In contrast to the wide variation in urine assay results, direct RIA of 6-keto-$PGF_{1\alpha}$ in Krebs-Henseleit medium from a perfused organ yielded high correlation of results, comparing the four antisera (Table VI). Thus, all of the antisera appear adequate for this type of in vitro study.

DISCUSSION

The standard for identifying and quantitating eicosanoids in biological fluids is GC—MS. The traditional method of fragmentation with electron impact has been used successfully to measure urinary 6-keto-$PGF_{1\alpha}$ [2, 8, 16], and this technique has been used to validate urinary 6-keto-$PGF_{1\alpha}$ RIAs [2, 8]. Recently, these GC—MS methods have been modified to increase sensitivity by use of NICI which reduces fragmentation, providing higher intensity of the high mass fragments [9]. The combination of NICI-MS with high-resolution capillary column GC and SIM provides sensitivity for urinary 6-keto-$PGF_{1\alpha}$ of about 2 pg [9]. This technique is the reference method; however, the expensive equipment and specialized personnel are not readily available to most laboratories. In contrast, RIA is a widely available, inexpensive and rapid technique. Because of repeated situations of suprious prostaglandin measurements on human plasma by RIA [17], it is essential to validate such assays with a reference technique. In the current study, GC—NICI-MS was used as the reference standard for comparison of antisera and chromatographic techniques for 6-keto-$PGF_{1\alpha}$ RIA.

Four antisera to 6-keto-$PGF_{1\alpha}$ obtained from different research laboratories were compared in this study. All had very similar cross-reactivity to primary prostaglandins, to TxB_2, and to the major urinary metabolites of 6-keto-$PGF_{1\alpha}$ (Table I). Assay of 6-keto-$PGF_{1\alpha}$ from Krebs-Henseleit medium of an isolated perfused rabbit colon yielded very high correlation of RIA results among the four antisera (Table VI). However, the RIAs of human urinary 6-keto-$PGF_{1\alpha}$ revealed marked differences with varying chromatographic systems and antisera.

Direct RIA of urine without extraction or chromatography yields results that are several fold higher than those of GC—NICI-MS. The higher results cannot be explained by cross-reactivity to the major urinary metabolites of 6-keto-$PGF_{1\alpha}$. Instead, there are a myriad eicosanoid metabolites in urine that

may potentially bind to these antisera. It is likely that some unidentified fatty acid metabolites co-migrate with 6-keto-$PGF_{1\alpha}$ on various chromatographic systems. Our study suggests that none of the standard chromatographic systems completely excludes these products. In addition, the varying results with the different antisera suggest that the different 6-keto-$PGF_{1\alpha}$ antisera bind to different contaminants despite very similar cross-reactivity testing to standard eicosanoids. There are numerous other chromatographic systems useful in purifying 6-keto-$PGF_{1\alpha}$ from urine, and it is possible that some of these systems may prove advantageous for RIA. However, it is our conclusion that the chromatographic system should be individually matched to each 6-keto-$PGF_{1\alpha}$ antiserum based on comparison to a reference technique. With this type of validation testing, RIA methods are available that are useful for studies of human urinary 6-keto-$PGF_{1\alpha}$.

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REFERENCES

1 T. Linne, E. Oliw and A. Aperia, Acta Physiol. Scand., 112 (1981) 149.
2 C. Patrono, F. Pugliese, G. Ciabattoni and P. Patrignani, J. Clin. Invest., 69 (1982) 231.
3 J. Nadler, R.D. Zipser, R. Coleman and R. Horton, J. Clin. Endocrinol. Metab., 56 (1983) 1260.
4 T.W. Wilson, F.A. McCauley and J.M. Tuchek, J. Chromatogr., 306 (1984) 351.
5 D. Benzoni, M. Vincent, G. Cuisinaud and J. Sassard, Clin. Chim. Acta, 126 (1982) 283.
6 W. Siess and F. Dray, J. Lab. Clin. Med., 99 (1982) 388.
7 B. Scherer, S. Fischer, W. Siess and P.C. Weber, Prostaglandins, 23 (1982) 41.
8 S. Fischer, B. Scherer and P.C. Weber, Biochim. Biophys. Acta, 710 (1982) 493.
9 S.E. Barrow, K.A. Waddell, M. Ennis, C.T. Dollery and I.A. Blair, J. Chromatogr., 239 (1982) 71.
10 J. Patterson, H. Kao and R.D. Zipser, Clin. Res., 32 (1984) 76.
11 W.S. Powell, Prostaglandins, 20 (1980) 947.
12 R.D. Zipser, T. Little, W. Wilson and R. Duke, J. Clin. Endocrinol. Metab., 53 (1981) 522.
13 A.R. Whorton, K. Carr, M. Smigel, L. Walker, K. Ellis and J.A. Oates, J. Chromatogr., 163 (1979) 64.
14 I. Alam, K. Ohuchi and L. Levine, Anal. Biochem., 93 (1979) 339.
15 R.D. Zipser, G.H. Radvan, I.J. Kronborg, R. Duke, and T. Little, Gastroenterology, 84 (1983) 697.
16 J.C. Frolich, B. Rosenkranz, W. Kitajuna, C. Fischer and L. Walker, in P.J. Lewis and J. O'Grady (Editors), Clinical Pharmacology of Prostacyclin, Raven Press, New York, 1981, p. 53.
17 E. Granstrom and H. Kindahl, in J.C. Frolich (Editor), Advances in Prostaglandin and Thromboxane Research, Vol 5, Raven Press, New York, 1978, p. 119.