Journal of Chromatography, 419 (1987) 103–111
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3682

COMPARATIVE STUDY OF SOLID-PHASE AND LIQUID-PHASE EXTRACTION TECHNIQUES FOR ISOLATION OF PHOSPHOLIPIDS FROM PLASMA

HASSAN SALARI

Department of Medicine, University of British Columbia, Vancouver General Hospital Research Institute, 2660 Oak Street, Vancouver, British Columbia V6H 3Z6 (Canada)

(First received November 12th, 1986; revised manuscript received March 1st, 1987)

SUMMARY

A liquid–liquid extraction technique and six solid absorbents, silica gel, octadecyl silica (C_{18}), XAD-2, XAD-4, XAD-7 and XAD-8, were compared as effective tools for extraction of phosphatidylcholine (Ptd C), phosphatidylethanolamine (Ptd E), phosphatidylinositol (Ptd I) and phosphatidylserine (Ptd S) from plasma. Using liquid–liquid extraction the recovery of the four phospholipids was in the order of 60% in organic phase and 20% in the liquid interface. Neither silica gel nor C_{18} materials significantly adsorbed phospholipids from plasma. Amberlite resins were more selective for removal of phospholipids. A recovery of greater than 85% was obtained for Ptd C, Ptd I and Ptd S when XAD-7 or XAD-8 were used as adsorbents. A recovery of approximately 90% for Ptd E was obtained when XAD-2 or XAD-4 were used as adsorbents. A solvent mixture of isopropanol–acetonitrile (1:1) was found to be the most effective eluent for removal of phospholipids from amberlite polymeric resins. These results suggest that resins could be used as an extracting tool for removal of phospholipids from body fluids.

INTRODUCTION

Up until the present date the techniques for extraction of phospholipids from biological fluids have employed a procedure known as liquid–liquid extraction. In liquid–liquid extractions, samples are mixed with a non-aqueous organic solvent such as chloroform and after separation of phases, phospholipids are recovered in the organic phase [1–5]. Among the existing liquid–liquid extraction techniques, there are two commonly used for extraction of phospholipids [2,5]. However, both techniques have major disadvantages, such as employment of large volumes of solvents, and are too time-consuming, which make them inconvenient for routine extraction. In addition, emulsion formation between aqueous and organic layers often prevents phase separation and may lead to loss of material.

0378-4347/87/$03.50 © 1987 Elsevier Science Publishers B.V.

Liquid–solid extractions employ solid adsorbents such as silica, octadecylsilane silica or Amberlite resins, from which adsorption of a compound from the liquid phase onto the adsorbent material and subsequent desorption may result in different recoveries depending on the type of adsorbent and eluting solvent utilized. Both silica and octadecylsilane silica have successfully been utilized to extract unsaturated lipids, particularly the arachidonic acid metabolites from biological fluids [6–10]. The acrylic ester copolymers XAD-7 and XAD-8 have also been used to extract lipids and various drugs from biological fluids [11–14]. Furthermore, the divinylbenzene–polystyrene polymers XAD-2 and XAD-4 have been used to extract drugs, steroids, arachidonic acid products [15–20] and alkylacetylglycerophosphocholines]21] from biological fluids.

The purpose of this study was to investigate the ability of these solid adsorbents to extract phospholipids from plasma and to find an alternative method to liquid–liquid extraction that would provide excellent recoveries with a minimum of manipulation.

EXPERIMENTAL

Chemicals

Amberlites XAD-2, XAD-4, XAD-7 and XAD-8 (50–100 μm) were obtained from Serva Feinbiochemica (Canadian supplier, Terochem Labs., Edmonton, Canada) and 1 g (dry mass) was packed into 10-ml polypropylene syringes. Silica and C_{18} Sep-Paks were purchased from Waters Assoc. (Milford, MA, U.S.A.). The radiolabelled phospholipids phosphatidylcholine [1-stearoyl-2-[1-^{14}C]*arachidonyl (50 mCi/mmol)*], *phosphatidylethanolamine* [1-stearoyl-2-[1-^{14}C]arachidonyl (50 mCi/mmol)] and phosphatidyl-[U-^{14}C]serine [1,2-dioleoyl (20–30 mCi/mmol)] were purchased from Amersham Radiochemicals (Oakville, Ontario, Canada). The synthetic non-radiolabelled phospholipids phosphatidylcholine (Ptd C), phosphatidylethanolamine (Ptd E), phosphatidylinositol (Ptd I) and phosphatidylserine (Ptd S) were purchased from Sigma (St. Louis, MO, U.S.A.). Silica thin-layer chromatographic (TLC) plates and Hibar silica gel columns (250 mm×4.0 mm I.D., 5 μm particle size) were obtained from E. Merck (Canadian supplier, Mandel Scientific, Rockwood, Canada). HPLC-grade solvents were purchased from Fisher Scientific (Vancouver, Canada) and were used as received.

Extraction procedure

Fresh human plasma (1 ml) (heparin anticoagulant) was spiked with 10^5 dpm (1 μg) of Ptd C, Ptd E, Ptd I or Ptd S in 2 μl of isopropanol–hexane (1:1). The plasma was diluted with 2 vols. of phosphate-buffered saline (PBS) and the mixture was acidified to pH 5 with hydrochloric acid. After centrifugation of the acidified solution (3000 *g*, 10 min), the samples were passed through either of the above solid adsorbents (silica, C_{18}, XAD-2, XAD-4, XAD-7 or XAD-8) or mixed with methanol and chloroform for the purpose of liquid–liquid extraction according to Bligh and Dyer [5]. The distribution of phospholipids was monitored by measurement of radioactivity in each manipulating step. For solid-phase

extractions, all columns were allowed to elute at a flow-rate of about 0.5 ml/min. The plasma eluates were collected and the amount of unretained phospholipids were determined by the presence of radioactivity in the eluates. The columns were then washed with 5 ml of water three times and radioactivity was monitored in each individual 5-ml eluate. The phospholipids were finally eluted from the columns with either 5 ml isopropanol–acetonitrile (1:1) four times or with various solvent mixtures (see Table IV) at a flow-rate of about 1 ml/min. Radioactivity was monitored in each individual 5-ml eluate. The elutions were carried out until radioactivity was no longer detected. Throughout the study, radioactivity was monitored by measuring a fraction of eluates in a beta-liquid scintillation counter.

Thin-layer chromatography

Phospholipids were applied to the non-activated silica gel plates in chloroform–methanol (2:1) and developed with chloroform–methanol–glacial acetic acid–water (50:25:8:2). The developing chamber was saturated with the solvent vapor. After drying of the plates 0.5-cm bands were scraped off. The compounds were eluted from the silica gel with chloroform–methanol–water (1:2:0.8) for 30 min at room temperature and centrifuged at 300 *g* for 15 min. An aliquot of the supernatant was counted to determine the radioactivity.

High-performance liquid chromatography

High-performance liquid chromatography (HPLC) was performed on a silica gel column (Hibar, 250 mm$\times$4.6 mm, 5 μm), using a Shimadzu Model LC-6A dual-pump system, which was connected to a variable UV–visible detector (210 nm) and a Gilson Model FC-80K fraction collector. Phospholipids individually (1 μg) or together were dissolved in 20 μl of isopropanol–hexane (1:1) and injected onto the column with a Rheodyne injector fitted with a 20-μl sample loop. Analysis was performed using a gradient from 100% solvent A to 100% solvent B at 50°C at a flow-rate of 1 ml/min. The composition of solvent A was hexane–isopropanol–water–tetrahydrofuran (THF) (48:49:2.5:0.5) and solvent B was hexane–isopropanol–water–THF–5 *M* sodium hydroxide (45:45:8:2:0.005). Phospholipids were monitored at 210 nm. Fractions (1 ml) were collected and aliquots were counted in a beta-liquid scintillation counter. The radioactive purity was determined to be greater than 95% as judged by TLC.

RESULTS

The recoveries of Ptd C, Ptd E, Ptd I and Ptd S from plasma using liquid–liquid extraction were 66$\pm$8.5, 60$\pm$6.5, 59$\pm$7 and 65$\pm$9%, respectively. Approximately 5–13% of radioactivity was found in the protein pellet after acidification and centrifugation. Ptd E exhibited the lowest and Ptd I the highest tendency toward trapping by denaturated protein. Upon extraction the aqueous phase did not contain any significant amounts of radioactivity, whereas the inter-face band between the aqueous and organic phases contained significant amounts of radioactivity: 18$\pm$4, 27$\pm$4.5, 22$\pm$6.5 and 20$\pm$3.5% for Ptd C, Ptd E, Ptd I and Ptd S, respectively. This was the main reason for loss of radioactivity upon extraction. When

TABLE I

RECOVERY OF PHOSPHOLIPIDS FROM 1 ml PLASMA USING SILICA GEL OR C_{18} MATERIALS AS ADSORBENTS

Step	Adsorbent	Eluent	Elution volume (ml)	Recovery (mean ±S.D., $n=5$) (%)			
				Ptd C	Ptd E	Ptd I	Ptd S
1	Silica gel	Plasma	3	28±3.5	51 ±9	63 ± 8.5	65±11
	C_{18}			60±8	54 ±7.5	71 ±10.5	49± 6.5
2	Silica gel	Water	5	15±4	26 ±5.5	19 ± 4	16± 3
	C_{18}			27±5.5	38 ±9	18 ± 3	31± 4
3	Silica gel	Water	5	2±0.5	1 ±0.5	3 ± 1	4± 1.5
	C_{18}			4±1	8 ±2.5	1.5± 0.5	2± 0.5
4	Silica gel	Water	5	0	0	0	0
	C_{18}			0	0	0	0
5	Silica gel	Isopropanol–acetonitrile (1:1)	5	34±5	13 ±3.5	8 ± 2	7± 2.5
	C_{18}			7±1	4.5±1	3 ± 0.5	12± 3
6	Silica gel	Isopropanol–acetonitrile (1:1)	5	21±6.5	5 ±1	2 ± 0.5	0
	C_{18}			0.5	0	0	1± 0.5
7	Silica gel	Isopropanol–acetonitrile (1:1)	5	1±0.5	0	0	0
	C_{18}			0	0	0	0
8	Silica gel	Isopropanol–acetonitrile (1:1)	5	0	0	0	0
	C_{18}			0	0	0	0

solid adsorbents were used for extraction, various recoveries were obtained for all the phospholipids tested. Table I shows the recovery of the four phospholipids from plasma using silica gel or C_{18} materials as adsorbents. Silica gel retained approximately 50% of Ptd C, 18% of Ptd E, 10% Ptd I and only 7% of Ptd S. Most of the radioactivity was coeluted with plasma or subsequent water washes. Similarly, C_{18} materials did not retain phospholipids from plasma samples significantly. As can be seen in Table I, most of the radioactivity was detected from plasma or water eluates and only 5–10% in the organic eluate (isopropanol–acetonitrile). When XAD-2 or XAD-4 resins were used, approximately 80% of all the phospholipids were retained by these materials (Table II). Both XAD-2 and XAD-4 appeared to be very effective adsorbents for these phospholipids, and were the best for Ptd E. A recovery of approximately 90% for Ptd E using XAD-2 or XAD-4 as adsorbent was obtained. XAD-4 was slightly less effective than XAD-2 in retaining Ptd C or Ptd I (Table II). Xad-7 and XAD-8, which are the medium polar resins were also very effective for retaining Ptd C, Ptd I and Ptd S from plasma, but less effective for Ptd E. Table III shows the recoveries of Ptd C, Ptd E, Ptd I and Ptd S from plasma using XAD-7 or XAD-8. The XAD-7 column retained approximately 87% of Ptd C, 79% of Ptd E, 89% of Ptd I and 93% of Ptd S from plasma. Likewise, XAD-8, a resin chemically similar to XAD-7 but with a smaller pore size and a larger surface area retained approximately 90% Ptd C, 65% Ptd E, 91% Ptd I and 87% Ptd S from plasma. Since XAD-2 was found to be the most effective solid adsorbent material for extraction of Ptd E from plasma and XAD-8 the most effective for Ptd C and Ptd I, a mixed resin column was developed. Table IV shows that the combination column of XAD-2

TABLE II

RECOVERY OF PHOSPHOLIPIDS FROM 1 ml PLASMA USING XAD-2 TO XAD-4 AS ADSORBENTS

Step	Adsorbent	Eluent	Elution volume (ml)	Recovery (mean ± S.D., $n=5$) (%)			
				Ptd C	Ptd E	Ptd I	Ptd S
1	XAD-2	Plasma	3	7 ±2	2.5±0.5	8± 2.5	9± 1.5
	XAD-4			15 ±3	5 ±1	11± 1.5	6± 1.5
2	XAD-2	Water	5	3.5±1	0.5	2± 0.5	3± 0.5
	XAD-4			4 ±0.5	3.5±0.5	5± 0.6	7± 2
3	XAD-2	Water	5	0	0	0	0
	XAD-4			0	0	0	0
4	XAD-2	Water	5	0	0	0	0
	XAD-4			0	0	0	0
5	XAD-2	Isopropanol-	5	29 ±6.5	38 ±5.5	41± 7	30±10
	XAD-4	acetonitrile (1:1)		45 ±8	38 ±7	28± 7	43± 8.5
6	XAD-2	Isopropanol-	5	45 ±7.5	47 ±8	46± 5.5	49± 8
	XAD-4	acetonitrile (1:1)		31 ±5.5	41 ±9.5	45±11.5	39± 5
7	XAD-2	Isopropanol-	5	11 ±4	9 ±3.5	7± 2	4± 0.5
	XAD-4	acetonitrile (1:1)		3 ±1	8 ±2	12± 3	2± 0.5
8	XAD-2	Isopropanol-	5	0	0	0	0
	XAD-4	acetonitrile (1:1)		0	0	0	0

TABLE III

RECOVERY OF PHOSPHOLIPIDS FROM 1 ml PLASMA USING XAD-7 OR XAD-8 AS ADSORBENTS

Step	Adsorbent	Eluent	Elution volume (ml)	Recovery (mean ± S.D., $n=5$) (%)			
				Ptd C	Ptd E	Ptd I	Ptd S
1	XAD-7	Plasma	3	5.5±2	24± 8	3 ± 0.5	8±2
	XAD-8			3 ±0.5	17± 2.5	5 ± 1.5	2±0.5
2	XAD-7	Water	5	1 ±0.5	3± 1	0	5±1.5
	XAD-8			0	8± 3	1.5± 0.5	0
3	XAD-7	Water	5	0	0	0	0
	XAD-8			0	0	0	0
4	XAD-7	Water	5	0	0	0	0
	XAD-8			0	0	0	0
5	XAD-7	Isopropanol-	5	30 ±5.5	58±11	42 ± 6	52±8.5
	XAD-8	acetonitrile (1:1)		48 ±7.5	40± 6	20 ± 3	35±4.5
6	XAD-7	Isopropanol-	5	55 ±8	20± 4	40 ±10	41±7
	XAD-8	acetonitrile (1:1)		37 ±8	23± 3	64 ± 5.6	51±0.5
7	XAD-7	Isopropanol-	5	2 ±0.5	1± 0.5	7.5± 1.5	0
	XAD-8	acetonitrile (1:1)		5 ±1	2± 0.5	7 ± 1	1±0.5
8	XAD-7	Isopropanol-	5	0	0	0	0
	XAD-8	acetonitrile (1:1)		0	0	0	0

TABLE IV

RECOVERY OF PHOSPHOLIPIDS FROM 1 ml PLASMA USING A COMBINATION OF XAD-2 AND XAD-8 ADSORBENTS

Eluent	Elution volume* (ml)	Recovery (mean ± S.D., $n=5$) (%)			
		Ptd C	Ptd E	Ptd I	Ptd S
Methanol	5	45 ± 3	26 ± 2	15 ± 6	11 ± 2.5
	10	68 ±11	48 ± 8.5	38 ±10	35 ± 6.5
	20	72 ± 8	59 ± 7	40 ± 8.5	38 ± 7
Ethanol	5	33 ± 2.5	31 ± 6.5	26 ± 7.5	33 ± 4.5
	10	70 ± 7	55 ± 8.5	42 ±12	55 ± 4.5
	20	75 ±10	63 ±10	51 ± 8	68 ± 5
Isopropanol	5	41 ± 5.5	33 ± 8	39 ± 7	25 ± 8
	10	66 ± 8.5	65 ± 5.5	55 ± 9.5	52 ± 8
	20	69 ± 8	61 ± 7	61 ± 6.5	70 ± 8.5
Acetonitrile	5	31 ± 4.5	20 ± 3	21 ± 4.5	11 ± 3
	10	58 ± 9	63 ± 8	43 ± 8	43 ± 8.5
	20	57 ±12	80 ± 7.5	65 ± 5	59 ± 7
Hexane	5	0	0	0	0
	10	1	0	2 ± 0.5	0
	20	1.5± 0.5	0	1	0
Chloroform	5	6 ± 2	0	0	0
	10	25 ± 7	5 ± 1	0	5 ± 1.5
	20	30 ± 2.5	6.5± 1.5	1.5± 0.5	5.6± 1.5
Isopropanol-hexane (1:1)	5	17 ± 5	11 ± 0.5	1 ± 0.5	17 ± 4
	10	39 ±11	23 ± 4.5	12 ± 3	19 ± 3.5
	20	45 ± 5.5	20 ± 5	10 ± 4	22 ± 9
Isopropanol-chloroform (1:1)	5	21 ± 6	20 ± 1.5	0	17 ± 4
	10	51 ±12	36 ± 2	2 ± 0.5	23 ± 6.5
	20	53 ± 7	45 ± 8	4 ± 1	39 ±11
Isopropanol-acetonitrile (1:1)	5	31 ± 4	38 ± 7	49 ± 6.5	35 ± 6
	10	92 ± 8.5	82 ± 9.5	87 ± 9	83 ±14
	20	95 ± 7	82 ±12	98 ±13.5	88 ± 8.5
Acetonitrile-methanol (1:1)	5	29 ± 5	23 ± 3.5	18 ± 3.5	29 ±11
	10	80 ± 7	75 ± 8	39 ± 8	46 ± 6.5
	20	82 ± 8.5	79 ± 6.5	56 ± 6.5	51 ± 8

*The given elution volume was obtained in one single step collection.

and XAD-8 removed most of the four phospholipids from plasma. However, the elution of these phospholipids from the column was dependent upon both the volume and composition of the eluent. Both highly polar and non-polar solvents were less effective in removal of phospholipids from the combination column. The most effective solvent for all of the four phospholipids was found to be acetonitrile–isopropanol (1:1) with an elution volume of 10–20 ml. Although XAD-7 alone turned out to be more effective than XAD-8 for the removal of Ptd E, no

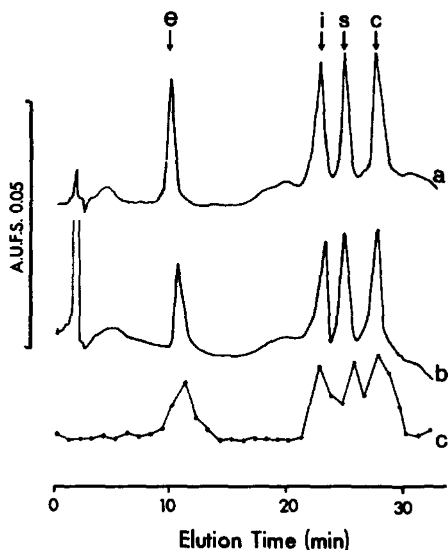

Fig. 1. Normal-phase HPLC analysis of a mixture of Ptd E, Ptd C, Ptd I and Ptd S. (a) Compounds (1 μg) before extraction with resin column. (b) Compounds (1 μg) after extraction with a combination of XAD-2 and XAD-8 resin. (c) Radioactivity distribution in the sample extracted with resins. Absorbance at 210 nm. Peaks: e = Ptd E; c = Ptd C; i = Ptd I; s = Ptd S.

significant difference was noticed in the recovery of Ptd E when a column of XAD-2 and XAD-7 in combination was used, or when a column of XAD-2 and XAD-8 in combination was used. The combined column of XAD-2 and XAD-8 was preferred because of its high flow-rate.

In order to determine whether the extraction of phospholipids by Amberlite resins provided clean samples for high-performance liquid chromatography (HPLC) and UV spectrophotometry, 10^5 dpm of ^{14}C-labelled and 1 μg of Ptd C, Ptd E, Ptd I and Ptd S were mixed and added to 3 ml of PBS containing 1% human serum albumin (free fatty acid). The sample was extracted with a combination column and eluted with 15 ml of acetonitrile–isopropanol (1:1). After evaporation of the eluent, the residue was dissolved in 20 μl of hexane–isopropanol (1:1) and injected onto a silica HPLC column. Fig. 1 shows the separation of Ptd E, Ptd I, Ptd S and Ptd C before and after extraction. As can be seen XAD resins did not add impurities in any significant quantity to the extraction residue.

DISCUSSION

The aim of this investigation was to determine the effectiveness of solid-phase extraction for the recovery of phospholipids from biological fluids. Solid-phase extraction has proven to be a useful tool for extraction of a variety of compounds and molecules of various origin. However, phospholipids are only extracted by means of liquid–liquid extraction. Most liquid–liquid extractions either lack the required specificity or are tedious and time-consuming. One of the most impor-

tant disadvantages of liquid–liquid extraction is the formation of an emulsion between the liquid phases, which can result in the considerable loss of sample. In this study, the effectiveness of the most common adsorbents were compared with liquid–liquid extraction. Attempts were made to maximize the recovery of four major phospholipids by using several different solvent mixtures and volumes, until no further phospholipids remained uneluted. It became evident that the silica-based materials are not very useful for extraction of phospholipids as phospholipids were coeluted with protein. However, the resin-based adsorbents proved to be excellent in retaining phospholipids. Nevertheless, the type of resin was observed to be important for the recovery of various classes of phospholipids, as it was noticed that Ptd E is well retained by polystyrene-based resins, such as XAD-2 or XAD-4, whereas Ptd C, Ptd I or Ptd S were equally retained by acrylic polymer and polystyrene based resins. A number of organic solvents such as ethanol, isopropanol, acetonitrile, acetonitrile–isopropanol, etc. proved to be effective for desorption of phospholipids from the resin. However, a solvent mixture of acetonitrile–isopropanol was found to be the most effective for the removal of these compounds from resins. In addition, this solvent mixture appeared to provide the highest recovery with less eluent. In general, it was found that for elution of phospholipids from the resins, it is necessary to use large volumes of eluting solvent for a satisfactory recovery. The requirement of large elution volumes could perhaps be due to the slight shrinking or swelling of the resin.

Although the main objective of this investigation was to develop a solid-phase extraction technique for phospholipids, an HPLC system for separation and estimation of these compounds in low microgram range was also developed. The HPLC method reported in this study is a great improvement on the previous technique reported by Blank and Snyder [22] with respect to resolution of Ptd E. The technique reported by the above investigators was found to have a few disadvantages: their system does not separate Ptd E and Ptd I well and also the baseline shift as the concentration of water in the mobile phase increases. By addition of THF to the mobile phase, an excellent separation of Ptd E, Ptd I and Ptd S was obtained. THF also improved peak resolution which is one of the important problems in phospholipid HPLC. Furthermore, THF counteracted the baseline shift as the concentration of water increased. Addition of sodium hydroxide improved the separation of Ptd S and Ptd C significantly.

In conclusion, XAD resins proved to be an excellent tool for the extraction of phospholipids from biological fluids prior to HPLC analysis and could presumably be used prior to gas chromatography–mass spectrometry where sample purity is a key factor.

ACKNOWLEDGEMENTS

This work was supported by the B.C. Health Care Research Foundation (of which H.S. is a scholar) and B.C. Lung Association.

REFERENCES

1 J. Folch, I. Ascoli, M. Lees, J.A. Meath and F.N. Lebaron, J. Biol. Chem., 191 (1951) 833.

2 J. Folch, M. Lees and G.H.S. Stanley, J. Biol. Chem., 226 (1957) 497.
3 N.J. Dambergs, Fish. Res. Board Can., 13 (1956) 791.
4 W.J. Dyer and M.L.J. Morton, Fish. Res. Board Can., 13 (1956) 129.
5 E.G. Bligh and W.J. Dyer, Can. J. Biochem. Physiol., 37 (1959) 911.
6 S.A. Metz, M.E. Hall, T.W. Harper and R.C. Murphy, J. Chromatogr., 233 (1982) 193.
7 F.J. Zijlstra and J.E. Vincent, J. Chromatogr., 311 (1984) 39.
8 W.S. Powell, Prostaglandins, 20 (1980) 947.
9 J.R. Luderer, D.L. Riley and L.M. Demers, J. Chromatogr., 273 (1983) 402.
10 W.C. Pickett and M.B. Douglas, Prostaglandins, 29 (1985) 83.
11 R.C. Murphy, S. Hammarstrom and B. Samuelsson, Proc. Natl. Acad. Sci. U.S.A., 76 (1979) 4275.
12 L. Orning, E. Norin, B. Gustafsson and S. Hammarstrom, J. Biol. Chem., 261 (1986) 766.
13 G.G. Volpé and V.N. Mallet, Int. J. Environ. Anal. Chem., 8 (1980) 291.
14 G.G. Volpé and V.N. Mallet, J. Chromatogr., 177 (1979) 385.
15 J.T. Stewart, T.S. Reeves and I.L. Honigberg, Anal. Lett., 7 (1984) 1811.
16 R. Dixon, R. Evans, and T. Crews, J. Liq. Chromatogr., 7 (1984) 177.
17 H.L. Bradlow, Steroids, 11 (1968) 265.
18 K. Green, M. Hamberg, B. Samuelsson and J.C. Frolich, Adv. Prostaglandin Thromboxane Res., 5 (1978) 15.
19 J.D. White and D.P. Schwartz, J. Chromatogr., 196 (1980) 303.
20 H. Salari and S. Steffenrud, J. Chromatogr., 378 (1986) 35.
21 H. Salari, J. Chromatogr., 382 (1986) 89.
22 M.L. Blank and F. Snyder, J. Chromatogr., 273 (1983) 415.