Journal of Chromatography, 378 (1986) 35—44
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3064

COMPARATIVE STUDY OF SOLID PHASE EXTRACTION TECHNIQUES FOR ISOLATION OF LEUKOTRIENES FROM PLASMA

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(First received September 2nd, 1985; revised manuscript received December 17th, 1985)

SUMMARY

We have compared five commercially available adsorbent materials (i.e. C_{18} Sep-Pak, C_{18} J.T. Baker, Amberlites XAD-7, XAD-2 and XAD-4) for their applicability as effective tools for extraction of leukotrienes from plasma samples. Leukotriene C_4 (LTC_4) and B_4 (LTB_4) were selected as representatives of peptidic and non-peptidic leukotrienes, respectively. These leukotrienes were added to 1 ml of plasma and passed through columns containing the above described adsorbent materials. The recovery was determined for each material using different combinations of solvents. XAD-4 gave the highest recovery for LTB_4 (90%), whereas XAD-4 and XAD-2 gave identical recoveries for LTC_4 (90%) when an eluting solvent mixture of pyridine—water—dimethylformamide (50:45:5) was used. The efficiency of the other three solid adsorbent materials for leukotriene extraction were in order of decreasing magnitude, C_{18} J.T. Baker > XAD-7 > C_{18} Sep-Pak. XAD-7 was shown to be more efficient for LTB_4 than for LTC_4, whereas the octadecylsilane C_{18} materials gave approximately similar recoveries for both of the leukotrienes. In addition to very good extraction properties of XAD-4 and XAD-2 as compared to octadecylsilane silica, these solid adsorbent materials retained less plasma impurities than the C_{18} materials, giving cleaner chromatograms for leukotrienes extracted from plasma. Therefore, XAD-4 or XAD-2 are the best overall choice for extraction of leukotrienes from plasma for reversed-phase high-performance liquid chromatographic analysis.

INTRODUCTION

Classical techniques for extraction of prostaglandins from biological fluids and plasma are liquid—liquid or liquid—solid extractions. In liquid—liquid extractions, chloroform, diethyl ether or ethyl acetate are used. This normally provides the analyst with good to excellent recoveries [1]. However, the liquid—liquid extractions are time-consuming since multi-step extractions may

0378-4347/86/$03.50

be needed in some cases or large volumes of organic solvents may be necessary for an efficient extraction. Emulsion formation with some organic solvents can hinder phase separation and result in a poor recovery. In addition, liquid–liquid extraction has not yet been demonstrated to be efficient for all the leukotrienes, particularly the peptidic leukotrienes which are less soluble in non-polar organic solvents. Liquid—solid extractions employ solid adsorbents such as silica or octadecylsilane silica materials 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 utilized. The most commonly used octadecylsilane is the C_{18} Sep-Pak. This solid phase material has been proven to be very effective for extraction of prostaglandins [2] and leukotrienes [3] from biological fluids. Another octadecylsilane silica material available in disposable columns of different sizes distributed by J.T. Baker has also been found to accomplish the extraction of prostaglandins and lipoxygenase metabolites of arachidonic acid from buffer with good recoveries [4]. Good recoveries for lipoxygenase metabolites of arachidonic acid from biological fluids are also obtained using C_{18} materials as adsorbents in an on-line automated extraction high-performance liquid chromatographic (HPLC) system [5].

The medium polar Amberlite XAD-7, an acrylic ester copolymer, was earlier used to extract peptidic leukotrienes from biological fluids [6]. Amberlite XAD-2, a cross-linked divinylbenzene-polystyrene polymer, has been used to extract various drugs [7], drug conjugates [8], steroids [9], as well as prostaglandins from biological fluids [10]. Amberlite XAD-4, a resin chemically similar to XAD-2 but with a smaller pore size and a larger surface area, has been used to extract glucuronides and sulfates from aqueous solutions [11].

The purpose of this study was to investigate the ability of these five commercially available solid adsorbent materials as effective extraction tools for leukotrienes in plasma and to find a solvent mixture which would selectively elute the adsorbed leukotrienes from the solid phase with a good yield.

EXPERIMENTAL

*Materials**

[5,6,8,9,11,12,14,15-$^3H_8$]LTB_4 (100 Ci/mmol) and [14,15-$^3H_2$]LTC_4 (20—60 Ci/mmol) were purchased from Amersham (Oakville, Canada) and were purified by ion-exchange chromatography as described [12]. By rechromatography of aliquots of the purified material the radiochemical purity was judged

*Leukotriene B_4 (LTB_4): 5*S*,12*R*-dihydroxy-6,8,10,14-(*Z*,*E*,*E*,*Z*,)-eicosatetraenoic acid; leukotriene C_4 (LTC_4): 5*S*-hydroxy-6*R*,*S*-glutathionyl-7,9,11,14-(*E*,*E*,*Z*,*Z*)-eicosatetraenoic acid; leukotriene D_4 (LTD_4): 5*S*-hydroxy-6*R*,*S*-cysteinylglycyl-7,9,11,14-(*E*,*E*,*Z*,*Z*)-eicosatetraenoic acid; leukotriene E_4 (LTE_4): 5*S*-hydroxy-6*R*,*S*-cysteinyl-7,9,11,14-(*E*,*E*,*Z*,*Z*)-eicosatetraenoic acid: 15-HETE: 15*S*-hydroxy-5,8,11,13-(*Z*,*Z*,*Z*,*E*)-eicosatetraenoic acid; 12-HETE: 12*S*-hydroxy-5,8,10,14-(*Z*,*Z*,*E*,*Z*)-eicosatetraenoic acid; 5-HETE: 5*S*-hydroxy-6,8,11,14-(*E*,*Z*,*Z*,*Z*)-eicosatetraenoic acid.

being at least 98%. Non-radioactive leukotrienes were obtained as a generous gift from Dr. J. Rokach of the Merck Frosst Laboratory (Montreal, Canada). ω-Hydroxy-LTB_4, 15-HETE, 12-HETE, and 5-HETE were prepared from human lung parenchyma after stimulation with ionphore A23187 [13]. Sep-Pak® octadecylsilane silica cartridges were obtained from Waters Assoc. (Milford, MA, U.S.A.). Other octadecylsilane silica disposable columns (1 g weight) were obtained from J.T. Baker (Phillipsburgh, NJ, U.S.A.). Amberlites XAD-7, XAD-2 and XAD-4 resins, 50–100 μm, were obtained from Serva Feinbiochemica (Canadian supplier: Terochem Labs., Edmonton, Canada) and 1 g (dry weight) of each resin was packed into 6-ml polypropylene cartridges (J.T. Baker). Before use, the columns were washed with 15 ml of methanol and 15 ml of distilled water.

Solvents of HPLC-grade were purchased from Fisher Scientific and used as received. Pyridine and dimethylformamide (DMF), ACS-grade (Fisher Scientific) were distilled before use.

Extraction procedure

Plasma samples (1 ml) freshly prepared and spiked with either LTB_4 ($1.7 \cdot 10^5$ dpm, mass = 34 ng) or LTC_4 ($1.6 \cdot 10^5$ dpm, mass = 39 ng) in 10 μl of water–methanol (1:1). These plasma samples were diluted three times with Dulbecco phosphate-buffered saline and then the mixture was acidified to pH 4 with 1 *M* hydrochloric acid. The acidified solution was passed through a column packed with either of five above mentioned adsorbent materials and the columns were washed extensively with water until no more protein could be eluted (e.g. showing no precipitation with acetone, usually 30 ml of water per ml of plasma). The leukotrienes were eluted from the column with different solvents or solvent mixtures (see Results) at a flow-rate of approx. 1.5 ml/min, using a slight vacuum, then 200 μl of the extracted solution were counted in a beta-liquid scintillation counter and the recovery (%) and precision data were calculated as mean ± S.D.

Reversed-phase high-performance liquid chromatography

Reversed-phase HPLC was performed using a C_{18} Radial-Pak cartridge (100 × 8 mm I.D., 10 μm particle size, Waters Assoc.) as described [14]. The metabolites of arachidonic acid were detected by ultraviolet spectrophotometry at 280 and 229 nm for leukotrienes and monohydroxyeicosatetraenoic acids, respectively.

RESULTS

Table I shows the recoveries obtained for the extraction of LTB_4 and LTC_4 from plasma using C_{18} Sep-Pak. As indicated, the desorption of leukotrienes was dependent on the solvent mixture and the volume of eluting solvent. With 3 ml of eluting solvent, the solvent mixtures methanol–water (90:10) or ethanol–water (80:20) were more efficient in desorbing LTB_4 and particularly LTC_4 from the adsorbent than pure methanol or ethanol. However, when larger volumes (> 15 ml) of methanol or ethanol were used, no significant differences were observed for the elution of LTB_4 or LTC_4 from Sep-Pak. In addition,

TABLE I

RECOVERY OF LTB_4 AND LTC_4 FROM 1 ml PLASMA WITH C_{18} SEP-PAK AFTER USING DIFFERENT SOLVENT SYSTEMS AND SOLVENTS

Compound	Volume eluting solvent (ml)	Eluting solvent	Recovery (mean ± S.D., $n = 4$) (%)
LTB_4	3	Methanol	20.0 ± 3.7
		Methanol—water (90:10)	20.5 ± 1.5
		Ethanol	21.0 ± 2.7
		Ethanol—water (80:20)	25.0 ± 1.2
	5	Methanol	38.0 ± 2.6
		Methanol—water (90:10)	40.0 ± 1.5
		Ethanol	35.0 ± 3.8
		Ethanol—water (80:20)	39.5 ± 2.1
	15	Methanol	37.0 ± 3.5
		Methanol—water (90:10)	38.0 ± 1.3
		Ethanol	38.0 ± 2.6
		Ethanol—water (80:20)	41.0 ± 1.5
LTC_4	3	Methanol	18.0 ± 3.1
		Methanol—water (90:10)	26.3 ± 4.5
		Ethanol	13.5 ± 2.0
		Ethanol—water (80:20)	25.1 ± 3.5
	5	Methanol	26.0 ± 2.5
		Methanol—water (90:10)	36.8 ± 3.5
		Ethanol	23.2 ± 2.0
		Ethanol—water (80:20)	35.5 ± 2.3
	15	Methanol	33.0 ± 1.8
		Methanol—water (90:10)	35.5 ± 3.1
		Ethanol	30.6 ± 2.7
		Ethanol—water (80:20)	34.8 ± 3.5

Sep-Pak did not retain more than 40% of the added leukotrienes and the remaining 60% was coeluted mainly with plasma or water wash (data not shown). After 15 ml of eluting solvent, no more radioactivity was detected in the effluents, indicating all of the adsorbed leukotrienes were eluted with 15 ml of solvent. When C_{18} J.T. Baker was used to extract LTB_4 or LTC_4 from plasma, similar results to the Sep-Pak were obtained (Table II). Once again, 80% ethanol or 90% methanol showed to be more efficient than pure methanol or ethanol. Nevertheless, C_{18} J.T. Baker was found to be more efficient than C_{18} Sep-Pak, the recoveries for LTB_4 and LTC_4 were about 59 and 51%, respectively. The remaining unrecovered LTC_4 or LTB_4 was detected in the protein fraction as well as in the water wash.

Amberlite XAD-7 resin was found to be more efficient than the C_{18} materials in retaining LTB_4 from plasma, whereas less efficient in retaining LTC_4 (Table III). In addition, the recoveries of LTB_4 or LTC_4 were not significantly affected by changing the eluting solvent or solvent mixture. The remaining unrecovered

TABLE II

RECOVERY OF LTB_4 AND LTC_4 FROM 1 ml PLASMA WITH C_{18} J.T. BAKER AFTER USING DIFFERENT SOLVENT MIXTURES AND VOLUMES

Compound	Volume eluting solvent (ml)	Eluting solvent	Recovery (mean ± S.D., n = 4) (%)
LTB_4	3	Methanol	38.0 ± 1.5
		Methanol—water (90:10)	43.0 ± 2.2
		Ethanol	40.0 ± 2.0
		Ethanol—water (80:20)	40.0 ± 2.5
	5	Methanol	55.0 ± 4.1
		Methanol—water (90:10)	54.0 ± 3.2
		Ethanol	53.0 ± 3.0
		Ethanol—water (80:20)	55.0 ± 3.5
	15	Methanol	54.5 ± 3.5
		Methanol—water (90:10)	58.5 ± 2.1
		Ethanol	53.0 ± 4.8
		Ethanol—water (80:20)	59.0 ± 2.9
LTC_4	3	Methanol	28.0 ± 2.8
		Methanol—water (90:10)	45.0 ± 3.2
		Ethanol	20.0 ± 3.0
		Ethanol—water (80:20)	31.0 ± 2.3
	5	Methanol	32.8 ± 2.2
		Methanol—water (90:10)	49.5 ± 3.1
		Ethanol	30.0 ± 1.3
		Ethanol—water (80:20)	50.0 ± 4.5
	15	Methanol	48.8 ± 3.5
		Methanol—water (90:10)	51.3 ± 3.1
		Ethanol	48.0 ± 2.8
		Ethanol—water (80:20)	49.5 ± 3.0

leukotrienes were detected in the protein eluate as well as in the water wash. When XAD-4 was used, a completely different pattern of recoveries was obtained. As shown in Table IV, the XAD-4 retained a greater amount of LTB_4 from plasma than XAD-7 or the C_{18} materials. By using methanol or a mixture of methanol—water as the eluting solvents, approx. 75% of adsorbed LTB_4 was eluted in 15 ml of eluting solvent. However, when a solvent mixture of pyridine—water—chloroform or pyridine—water—DMF was used, up to 90% of LTB_4 was recovered in 15 ml of eluate. Pyridine alone, pyridine–water or pyridine–DMF was shown to be less efficient in desorbing LTB_4 from XAD-4. XAD-4 also retained LTC_4 strongly and the majority of the organic solvents or solvent mixtures tried were unable to desorb this compound. As Table IV demonstrates, the solvent mixtures which were able to desorb LTC_4 were pyridine—water (50:50), pyridine—water—chloroform (50:45:5), and pyridine–water—DMF (50:45:5). The most efficient solvent mixture for eluting LTC_4 from XAD-4 was pyridine–water—DMF (50:45:5) in which a recovery of approx. 91% was obtained.

TABLE III

RECOVERY OF LTB_4 AND LTC_4 FROM 1 ml PLASMA WITH 1 g XAD-7 AFTER USING DIFFERENT ELUTING-SOLVENT MIXTURES

Compound	Eluting solvent (elution volume 15 ml)	Recovery (mean ± S.D., n = 4) (%)
LTB_4	Methanol	65.0 ± 3.2
	Ethanol	68.0 ± 2.0
	Methanol—water (90:10)	68.0 ± 2.0
	Ethanol—water (80:20)	63.5 ± 2.3
	Pyridine—water (50:50)	65.3 ± 3.8
	Pyridine—water—DMF (50:45:5)	67.8 ± 3.0
	Pyridine—DMF (50:50)	55.0 ± 3.5
LTC_4	Methanol	35.0 ± 2.5
	Ethanol	32.5 ± 1.8
	Methanol—water (90:10)	34.2 ± 2
	Ethanol—water (80:20)	30.0 ± 2.5
	Pyridine—water (50:50)	36.0 ± 4.5
	Pyridine—water—DMF (50:45:5)	32.0 ± 4.2
	Pyridine—DMF (50:50)	28.0 ± 2.3

When XAD-2 was used to extract LTB_4 and LTC_4 from plasma almost identical results to XAD-4 were obtained (Table V) in which approx. 90% of LTB_4 or LTC_4 was recovered using 15 ml of pyridine- water- DMF (50:45:5).

In another series of experiments, the ability of these five solid adsorbent materials as efficient tools for the extraction of LTC_4 and LTB_4 using 15 ml of the most efficient solvent mixture for each adsorbent was compared. As shown in Table V, XAD_4 and XAD-2 were the best solid adsorbent materials for extraction of both LTC_4 and LTB_4 from plasma, followed by XAD-7 (LTC_4 recovery $< C_{18}$) $> C_{18}$ J.T. Baker $> C_{18}$ Sep-Pak.

In order to determine whether extraction of leukotrienes from plasma using XAD-4 provides a cleaner sample for HPLC and UV spectrophotometry, we added 100 ng of ω-hydroxy-LTB_4, LTB_4, LTC_4, LTD_4, LTE_4, 5-HETE, 12-HETE and 15-HETE, as well as 200 ng of prostaglandin B_2 (PGB_2) to 1 ml of plasma and then extracted with either XAD-4 or C_{18} J.T. Baker using the solvent mixtures as shown in Table V. The eluted materials from both solid adsorbents were evaporated under reduced pressure at 35°C and then the residues were dissolved in 1 ml of 50% methanol. The residues were analysed by reversed-phase HPLC. As demonstrated in Fig. 1, XAD-4 not only provided an excellent recovery for all the arachidonic acid metabolites, it also provided a cleaner sample extract (Fig. 1A) than the C_{18} adsorbents (Fig. 1B).

A disadvantage with the resins is the large elution volumes required for a satisfactory recovery, probably depending on a slight shrinking or swelling of the resin upon a change of the solvent. Furthermore, pyridine is toxic and smells, therefore it is recommended to work in a fume hood. We are currently investigating the possibility of a combination of solvents that will prevent shrinking with a retained excellent recovery which includes a decrease of the pyridine content or the employment of another nitrogen containing solvent.

TABLE IV

RECOVERY OF LTB_4 AND LTC_4 FROM 1 ml PLASMA WITH 1 g XAD-4 AFTER USING DIFFERENT SOLVENT MIXTURES

Compound	Eluting solvent (elution volume 15 ml)	Recovery (mean ± S.D., $n = 4$) (%)
LTB_4	Methanol	71.0 ± 3.6
	Methanol—water (90:10)	76.0 ± 4.5
	Ethanol	69.0 ± 4.2
	Ethanol—water (80:20)	75.0 ± 2.8
	Pyridine	63.0 ± 2.5
	Pyridine—water (50:50)	78.0 ± 3.5
	Pyridine—water—chloroform (50:45:5)	89.0 ± 2.8
	Pyridine—water—DMF (50:45:5)	92.0 ± 3.1
	Pyridine—DMF (50:50)	72.0 ± 2.8
LTC_4	Methanol	6.0 ± 1.0
	Methanol—water (90:10)	7.0 ± 1.2
	Methanol water (50:50)	6.7 ± 0.8
	Methanol—water—toluene (85:13:2)	7.0 ± 0.5
	Methanol—dimethylsulfoxide—water (80:10:10)	6.5 ± 1.0
	Methanol—water—DMF (85:10:5)	15.0 ± 2.5
	Ethanol	7.0 ± 1.0
	Ethanol—water (80:20)	8.0 ± 0.5
	Isopropanol	3.0 ± 0.5
	Isopropanol—water (90:10)	4.0 ± 1.0
	Acetonitrile	0
	Acetone	0
	Acetone—water (50:50)	1.2 ± 0.5
	Acetone—water (30:70)	0
	Acetone—water—DMF (50:45:5)	5.1 ± 0.5
	Chloroform	0
	Hexane	0
	Toluene	0
	Dimethylsulfoxide	0
	DMF	0
	DMF—water (50:50)	0
	DMF—pyridine (50:50)	4.0 ± 1.0
	Tetrahydrofuran	0
	Tetrahydrofuran—water (80:20)	8.0 ± 1.0
	Tetrahydrofuran—water (50:50)	9.0 ± 1.5
	Tetrahydrofuran—water (30:70)	10.0 ± 1.5
	Tetrahydrofuran—water (15:85)	3.0 ± 0.5
	Tetrahydrofuran—dimethylsulfoxide—water (30:10:60)	5.0 ± 0.5
	Pyridine	9.0 ± 1.0
	Pyridine—water (80:20)	40.0 ± 6.0
	Pyridine—water (50:50)	78.0 ± 4.0
	Pyridine—water (30:70)	51.0 ± 3.2
	Pyridine—water (10:90)	36.0 ± 2.0
	Pyridine—water—chloroform (50:45:5)	85.0 ± 2.5
	Pyridine—water—DMF (50:45:5)	91.0 ± 2.3

TABLE V

COMPARATIVE STUDY ON THE RECOVERY OF LTB_4 AND LTC_4 FROM 1 ml PLASMA WITH XAD-4, XAD-2, XAD-7, C_{18} J.T. BAKER AND C_{18} SEP-PAK AFTER USING 15 ml OPTIMAL SOLVENT MIXTURE FOR EACH ADSORBENT

Adsorbent	Recovery (mean ± S.D., n = 5) (%)		Eluting solvent
	LTB_4	LTC_4	
XAD-4	92.0 ± 3.1	90.0 ± 1.6	Pyridine—water—DMF (50:45:5)
XAD-2	88.0 ± 3.5	90.1 ± 1.7	Pyridine—water—DMF (50:45:4)
XAD-7	66.0 ± 5.1	32.0 ± 2.8	Methanol—water (90:10)
C_{18} J.T. Baker	58.0 ± 3.0	51.0 ± 2.5	Methanol—water (90:10)
C_{18} Sep-Pak	39.0 ± 2.5	34.0 ± 2.0	Methanol—water (90:10)

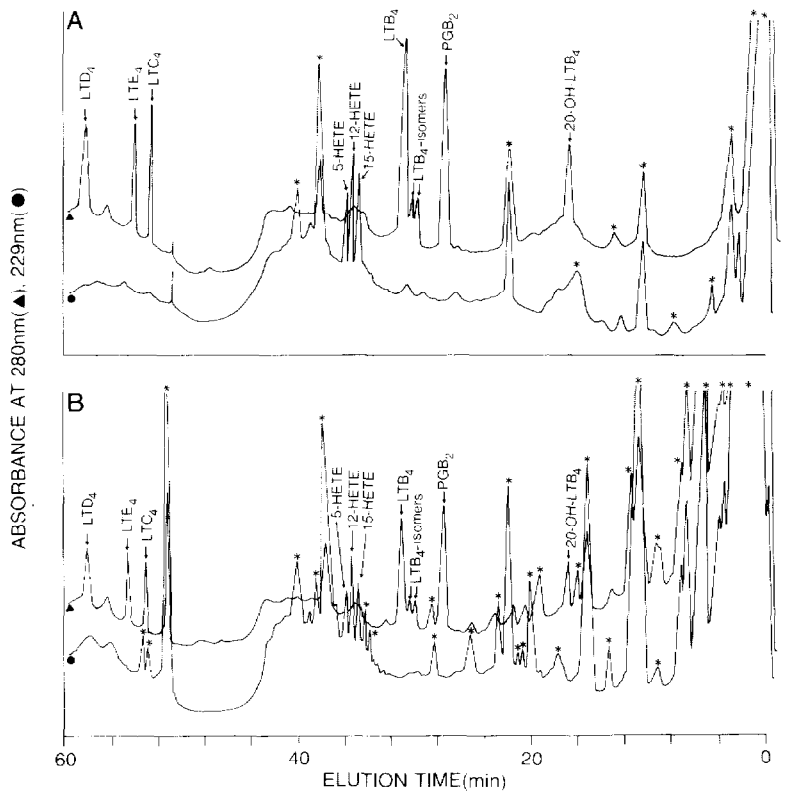

Fig. 1. Reversed-phase HPLC profiles obtained from plasma extracted standards of leukotrienes and monohydroxyeicosatetraenoic acid with XAD-4 (A) and with C_{18} J.T. Baker (B). The analysis was carried out on a Waters C_{18} Radial-Pak cartridge (100 × 8 mm I.D.; 10 μm particle size). The traces show the UV absorbances at 280 nm (▲) and 229 nm (●). The attenuation settings of UV spectrophotometers were 0.04 and 0.1 a.u.f.s. at 280 and 229 nm, respectively. (*) Plasma impurities.

DISCUSSION

The objective of this study was to compare five commercially available solid adsorbent materials for lipoxygenase metabolites of arachidonic acid, particularly the leukotrienes from plasma so that general guidelines could be

suggested to the analyst who wishes to measure leukotrienes in plasma. Attempts were made to maximize the recovery by using several different solvent mixtures, until no further leukotrienes remained uneluted. The three solid phase extraction systems C_{18} Sep-Pak, C_{18} J.T. Baker and XAD-7 have already been used to extract leukotrienes from biological fluids [4–6, 13, 15]. XAD-2, which is more hydrophobic than XAD-7, has been used for extraction of prostaglandins but not leukotrienes [10]. XAD-4, another hydrophobic resin, has not been used earlier to extract either prostaglandins or leukotrienes. However, it has been reported that this resin retains a variety of conjugated [11] and non-conjugated [16, 17] compounds so strongly that the use of methanol which is usually employed for the elution of adsorbed compounds, results in a poor recovery. In the view of this characteristic, its eventual usefulness for the extraction of leukotrienes from plasma was investigated.

Although an excellent recovery (90%) for prostaglandins from plasma with C_{18} Sep-Pak has been reported [3], nevertheless, our studies with Sep-Pak as a solid adsorbent material demonstrated that this system is unable to retain the leukotrienes to any larger extent. Poor recoveries for lipoxygenase metabolites of arachidonic acid using C_{18} Sep-Pak has also been reported by two other laboratories [18, 19]. The other C_{18} solid adsorbent material (C_{18} J.T. Baker) was shown to be better for the extraction of leukotrienes from plasma. Nevertheless, this system was also found insufficient for retaining a high percentage of leukotrienes from 1 ml of plasma. XAD-7 was also found to give poor recoveries of leukotrienes from plasma. The non-peptidic leukotrienes were retained better than the peptidic. The desorption of leukotrienes from XAD-7 was less dependent on the type of solvent or solvent mixture employed and thus similar recoveries for LTB_4 and LTC_4 were observed using different eluting solvents (Table III).

The best adsorbent materials for extracting leukotrienes from plasma were demonstrated to be XAD-2 or XAD-4. These two adsorbent materials retained almost all of the leukotrienes, polar as well as less polar, and the monohydroxy-eicosatetraenoic acids. Methanol and several other organic solvents or solvent mixtures (Table IV) were able to elute the retained non-peptidic leukotrienes. Although some organic solvents were more efficient than others at smaller volumes nevertheless, when the elution volume was increased, similar recoveries were obtained for all of them. Interestingly, the peptidic leukotrienes adsorbed so strongly on the XAD-4 or XAD-2 that none of the commonly used solvents were able to desorb them. Using methanol or ethanol and many other solvents (see Table IV), only very small amounts of peptidic leukotrienes were recovered. Similar results have been reported with sulfate or glucuronide conjugates [11]. The peptidic leukotrienes were desorbed from XAD-4 or XAD-2 only by a very limited number of solvent mixtures. The best solvent mixture was pyridine—water (50:50) buffered to pH 6.5 with acetic acid. The kind of mechanism involved in this process is not yet clear. The fact that pyridine—water (1:1) was found to constitute the near optimal mixture indicates that a balance has to be found between the solubility of LTC_4 in the eluting solvent and the interaction of the eluting solvent with the resin. To elucidate how nitrogen-containing solvents affect the elution, needs further experiments including other nitrogen-containing solvents because DMF—water (1:1) or DMF alone resulted in no elution at all of LTC_4.

However, it is noteworthy to mention that a majority of the solvents seemed to cause the XAD-4 or XAD-2 to shrink as indicated by an impaired flow-rate upon elution and thus might be an explanation for the relatively large elution volume. Chloroform and, to a certain extent, DMF were able to counteract the shrinkage of the resin and presumably resulting in a better penetration of the pores of the resin by the mobile phase. Therefore, when small amounts of chloroform or DMF were added to the pyridine—water mixture, the LTC_4 recovery in 15 ml of elution increased by a factor of about 10%.

XAD-4 as a material for solid phase extractions also proved to co-extract less impurities than C_{18} materials and thus might be used to clean biological samples prior to micro-bore chromatography where the sample loading capacity is limited. Therefore, we concluded that the XAD-4 or XAD-2 are the overall best choice for the extraction of leukotrienes and probably prostaglandins from aqueous solutions for reversed-phase HPLC. Further investigations are in progress to determine leukotriene recovery from larger volumes of human plasma, urine and synovial fluids.

ACKNOWLEDGEMENTS

We wish to thank Dr. M. Yeung and the B.C. Health Care Research Foundation for support in the preparation of this manuscript.

REFERENCES

1 E. Granstrom and H. Kindahl, in J.C. Frolich (Editor), Advances in Prostaglandins and Thromboxane Research, Vol. 5, Raven Press, New York, 1978, p. 119.
2 W.S. Powell, Prostaglandins, 20 (1980) 947.
3 H. Salari, I.W. DeVoe and W.S. Powell, Biochem. Biophys. Res. Commun., 104 (1982) 1517.
4 J.R. Luderer, D.L. Riley and L.M. Demers, J. Chromatogr., 273 (1983) 402.
5 W.C. Pickett and M.B. Douglas, Prostaglandins, 29 (1985) 83.
6 R.C. Murphy, S. Hammarstrom and B. Samuelsson, Proc. Natl. Acad. Sci. U.S.A., 79 (1979) 42.
7 J.T. Stewart, T.S. Reeves and I.L. Honigberg, Anal. Lett., 7 (1984) 1811.
8 R. Dixon, R. Evans and T. Crews, J. Liq. Chromatogr., 7 (1984) 177.
9 H.L. Bradlow, Steroids, 11 (1968) 265.
10 K. Greeen, M. Hamberg, B. Samuelsson and J.C. Frolich, in J.C. Frolich (Editor), Advances in Prostaglandin and Thomboxane Research, Vol. 5, Raven Press, New York, 1978, p. 15.
11 J.D. White and D.P. Schwartz, J. Chromatogr., 196 (1980) 303.
12 S. Steffenrud and P. Borgeat, in B. Samuelsson, R. Paloletti and P.W. Ramwell (Editors), Advances in Prostaglandin, Thomboxane and Leukotriene Research, Vol. 15, Raven Press, New York, 1985, p. 59.
13 H. Salari, P. Borgeat, M. Fournier, J. Hebert and G. Pelletier, J. Exp. Med., 162 (1985) 1904.
14 P. Borgeat, Prostaglandins, 27 (1984) 356.
15 H. Salari, Immunology, in press.
16 D.J. Pictrzyk and C.H. Chu, Anal. Chem., 49 (1977) 757.
17 D.J. Pictrzyk and C.H. Chu, Anal. Chem., 49 (1977) 860.
18 F.J. Zijlstra and J.E. Vincent, J. Chromatogr., 311 (1984) 39.
19 S.A. Metz, M.E. Hall, T.W. Harper and R.C. Murphy, J. Chromatogr., 233 (1982) 193.