Waters Lab Highlights

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Isolation of Platelet-Activating-Factor by Solid-Phase Extraction using Sep-Pak® Cartridges

Platelet-activating factor (1-O-alkyl-2-acetyl-*s,n*-glycero-3-phosphocholine) (PAF) is a naturally occurring phospholipid with a wide spectrum of biological activities. The isolation and analysis of PAF is difficult because it is produced in vivo in amounts five to six orders of magnitude less than the principal tissue and blood lipids, including phosphatidylcholine, phosphatidylethanolamine, cholesterol, and cholesterol ester. Direct extraction from biological tissues and fluids is unsatisfactory, because PAF cannot be selectively removed from the non-PAF lipids.

Researchers at Hoffmann-La Roche recently reported on the use of silica Sep-Pak cartridges for the enrichment and isolation of PAF from lipid extracts[1]. Lipid samples were dissolved in chloroform, and ~ 250µl were applied to the top surface of the Sep-Pak frit using a micropipette. The lipids were then eluted using the sequence of solvents described in the footnote to Table 1. Flow rate was controlled manually at about 30 ml/min at room temperature. It was found that PAF could be quantitatively eluted, along with other choline phospholipids, but free of neutral lipids, in 10 ml of methanol-chloroform-water (2:1:0.8, v/v). The PAF isolated using this technique retained full biological activity as measured by a receptor-binding assay. Table 1 shows the recoveries of lipids obtained from four fractions eluted from the Sep-Pak cartridge.

Table 1. Elution and Recovery of Lipids from Silica Sep-Pak Cartridges[1]

Lipid Class	Recovery[a] (%)			
	Fraction 1	Fraction 2	Fraction 3	Fraction 4
Neutral Lipid	99.8±0.5	0	0	0
Phosphatidylcholine	0	<0.1	99.2±0.7	0
Phosphatidylethanolamine	0	97.2±1.0	<0.1	<0.1
Phosphatidylinositol	0	98.3±0.9	>0.1	<0.1
Phosphatidylserine	0	62.3±0.9	37.1±0.8	<0.1
Phosphatidylglycerol	0	99.3±0.5	0	0
Cardiolipin	0	99.7±0.6	0	0
Sphingomyelin	0	<0.1	98.6+0.6	0
Lysophosphatidylcholine	0	<0.1	99.1±0.8	<0.1
Lyso-PAF	0	<0.1	98.3+0.5	<0.1
PAF	0	<0.1	100.2+2.4	0

[a]Results are mean ± S.D. (n ≥ 4) of each lipid class as assessed by internal radiolabeled lipid standards added to ≤ 5mg rat heart muscle tissue. Fraction 1 was eluted in 16 ml chloroform-acetic acid (100:1, v/v); fraction 2 was eluted in 5 ml methanol-chloroform (2:1,v/v); fractions 3 and 4 were each eluted in 10 ml volumes of methanol-chloroform-water (2:1:0.8,v/v).

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The method described by the authors provides a technique for enriching crude lipid extracts easily, inexpensively, and with excellent recoveries. In addition "the high. . . capacity of the method is noteworthy in comparison to the far (>50-fold) lower capacity of analytical TLC. The quantitative recovery of PAF in this solid-phase extraction system is also compelling, since PAF recovery from multistep column chromatography procedures may be as low as 30%.[1]"

A PAF-enriched fraction can be prepared in minutes, compared to several hours for conventional column chromatography. The authors concluded that ". . . solid phase extraction on pre-packed silica cartridges has several unique advantages over conventional chromatographic methods for PAF enrichment from lipid mixtures and thus constitutes an improvement over such methods.[1]"

Phospholipids like PAF are potent regulators of biological functions, and thus are of great interest to bioresearchers. This field represents a large potential growth opportunity for HPLC.

Reference

1. Janero, D. R., and Burghardt, C., *J. Chromatogr. Biomed. Applications*, **526**, (1990) 11-24.

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