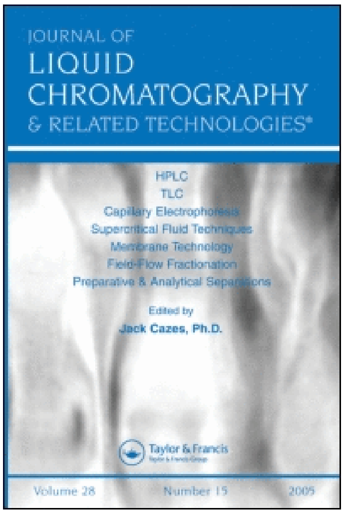

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Quantitative Analysis of Polyamines at Trace Levels by High Performance Liquid Chromatography in High Salt Solutions. Application to Seawater

C. Cann-Moisan[a]; J. Caroff[a]; A. Hourmant[b]; C. Videau[b]; F. Rapt[b]
[a] Laboratoire de Physiologie, Faculté de Médicine, Brest, Cédex, France [b] Laboratoire de Physiologie Vigitale, Faculté des Sciences, Brest, Cédex, France

To cite this Article Cann-Moisan, C. , Caroff, J. , Hourmant, A. , Videau, C. and Rapt, F.(1994) 'Quantitative Analysis of Polyamines at Trace Levels by High Performance Liquid Chromatography in High Salt Solutions. Application to Seawater', Journal of Liquid Chromatography & Related Technologies, 17: 6, 1413 — 1417

To link to this Article: DOI: 10.1080/10826079408013773

URL: http://dx.doi.org/10.1080/10826079408013773

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(6), 1413-1417 (1994)

QUANTITATIVE ANALYSIS OF POLYAMINES AT TRACE LEVELS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN HIGH SALT SOLUTIONS. APPLICATION TO SEAWATER

C. CANN-MOISAN[1*], J. CAROFF[1], A. HOURMANT[2],
C. VIDEAU[2], AND F. RAPT[2]
[1]*Laboratoire de Physiologie*
Faculté de Médicine, BP 815
Service "Lavoisier", CHU Morvan
29 285 Brest Cédex, France
[2]*Laboratoire de Physiologie Végétale*
Faculté des Sciences
Avenue Le Gorgeu
29 287 Brest Cédex, France

ABSTRACT

This paper presents an original method to determine polyamines (putrescine, spermidine and spermine) by HPLC in high salt solutions. Sample preparation consists in many steps such as lyophilisation and dansylation before analysis. This method offers the advantages to be reproducible, linear and sensitive, as example we applied this technique to seawater and determined polyamines at trace level.

INTRODUCTION

Ubiquity and physiological importance of the polyamines in living systems (animal and plant) explain the numerous methods described in the last few years for their analysis and quantification.

High performance liquid chromatography with fluorimetric detection is mainly used with O-phthaldehyde (1) or dansylchloride (2, 3) as fluorescent agents.

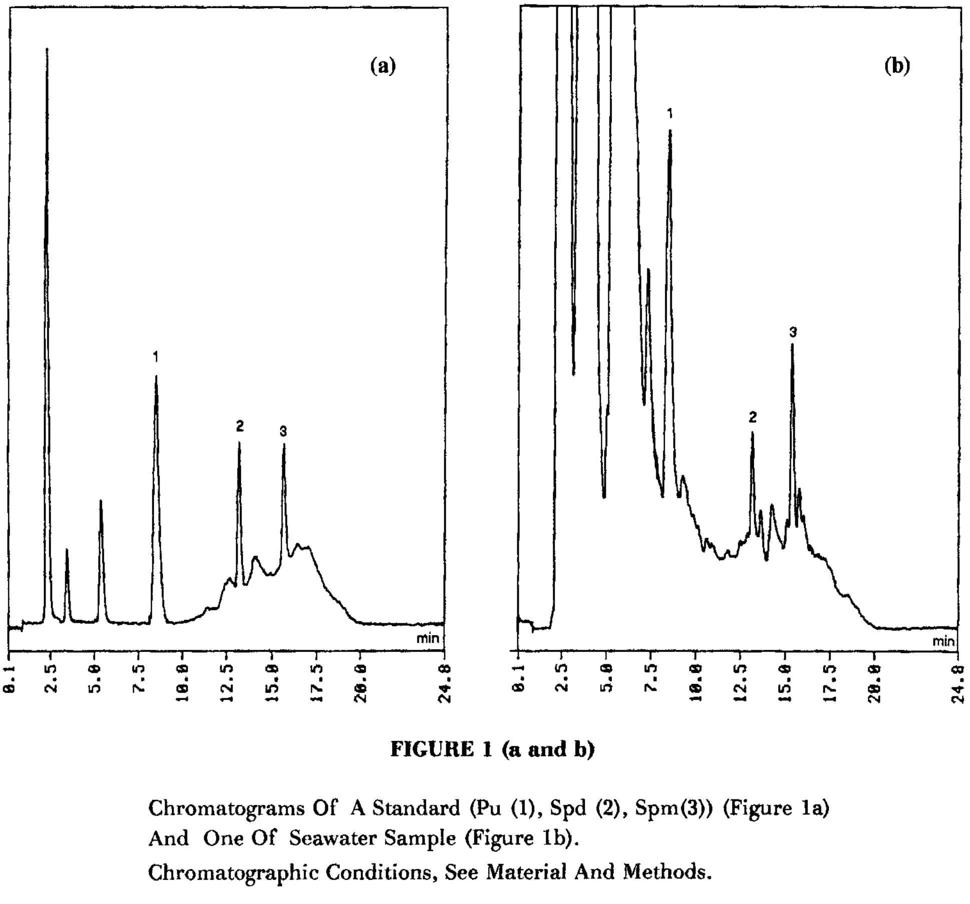

FIGURE 1 (a and b)

Chromatograms Of A Standard (Pu (1), Spd (2), Spm(3)) (Figure 1a) And One Of Seawater Sample (Figure 1b).

Chromatographic Conditions, See Material And Methods.

In this paper, we describe an original method to determine polyamines (Putrescine (Pu), Spermidine (Spd) and Spermine (Spm)) in high salt synthetic solutions (NaCl 35 o/oo). Many steps are necessary to prepare the extract before dansylation and separation on reversed phase column. In particular, the first and most important problem consists in removing salt without loss of polyamines.

In the present study we have applied this method to sea water and have detected free polyamines at trace levels.

REAGENTS AND SOLVENTS

Sodium hydrogenphosphate, sodium chloride, sodium carbonate, perchloric acid were of analytical reagent grade and benzene, acetone, acetonitrile, methanol were HPLC grade solvent (Merck, Darmstadt, Germany).

Dansyl chloride, putrescine, spermidine and spermine were purchased from Sigma (St Louis, MO, USA).

APPARATUS

The HPLC system consisted of two model 420 pump (Kontron) coupled with a high pressure mixer, an autosampler MSI660 (Kontron) with a 7110 Rheodyne injection valve fitted with a 20 ul loop.

Separation was achieved on Ultrasphere ODS column (250 x 4.6 mm ID, 5 um) protected by a Brownlee RP 18 guard column (30 x 4.6 mm ID 5 um). A gradient was realized in 25 min with two mobile phases A : (methanol-acetonitril 50 : 50 V/V) and B (NaH_2PO_4 0.01 M, pH 4.4) at a flow rate of 1.0 $ml.min^{-1}$: 5 min 20% B, 2 min 20% B to 11% B, 5 min 11% B to 0% B, 3 min 0% B to 20% B, 10 min 20% B. Solvents and mobile phases were filtered on 0.45 u (Millipore) before used.

Detection was accomplished using a spectrofluorimeter (SFM 25, Kontron) at an excitation wave length of 360 nm, and emission wave length of 510 nm. This HPLC system was controlled by a microcomputer Data system 450 (Kontron).

EXTRACTION PROCEDURE AND DANSYLATION

Synthetic mixtures with added amounts of Pu, Spd, Spm and seawater were treated in parallel.

5 ml NaCl 35 o/oo with Pu, Spd, Spm
or 5 ml seawater

Lyophilisation

Polyamine
extraction + 3 ml methanol

Centrifugation 4000 tr/min 10 min
Evaporation of the surpernatant under vacuum

+ 200 ul $HClO_4$ 5%
Dansylation + 200 ul Na_2CO_3 saturated
overnight in dark + 200 u l dansyl chloride (5 mg/ml in acetone)

Extraction with 2 ml C_6H_6 (twice one ml)

Evaporation under vacuum

+ 200 ul methanol
Filtration (0.45 um) and injection into the HPLC system

TABLE I

Pu	Spd	Spm
894 ± 237.2	203 ± 65.4	1050 ± 169.7

Polyamine Contents in Sea water Expressed in pg/ml (X ± SE), N = 5.

RESULTS AND DISCUSSION

The first step in the extraction procedure of polyamines consists of removing as much salt as possible from the high salt solution before dansylation. Indeed, this derivatization procedure requires favorable conditions of pH, reaction time and ionic strength. Lyophilisation is a good technique to remove water and replace it with methanol without any loss of polyamines because sodium chloride is less soluble in this solvent . The linearity of the procedure was tested from 0.015 ng to 0.5 ng injected, for Pu, Spd, and Spm in NaCl 35 o/oo solution. The linear regression equation are the following : Pu : $y = 59.49\ x - 0.364$ ($r = 0.995$), Spd : $y = 84.95\ x - 0.076$ ($r = 0.999$), Spm : $y = 66.74\ x - 0.138$ ($r = 0.999$). Polyamines were identified with the method of constant additions. No internal standard was used in reference to our previous paper which discussed the choice of diaminohexane (DAH) as internal standard (4). Figure 1 shows chromatograms of a standard mixture (Pu, Spd, Spm) (Figure 1a) and one of seawater (Figure 1b).

In the seawater tested in our study, polyamines are at trace level (Table I).

Intra assay coefficients of variation for sea water are the following (N = 5) Pu : CV = 0.26, Spd : CV = 0.51, CV = 0.36 ; these results are good when analysis is performed at such a sensitivity. Indeed, the limits of the detection were 5 pg, 5 pg, 1.5 pg injected for Pu, Spd and Spm respectively, with a signal to noise ratio of 2.

This method is reproducible and sensitive ; it enables to determine polyamines at trace levels in seawater.

It will be interesting to study polyamine concentrations in sea water samples drawn from different environments. Applications are numerous if there exists a relationship between the level of free polyamines in sea water and living activity.

REFERENCES

1. J.L. Corbin, B.M. Marsh, G.A. Peters. Plant Physiol. 90 : 434-439 (1989).

2. H.E. Flores, A.W. Galston. Plant Physiol. 69 : 701-706 (1982).

3. S. C. Minocha, R. Minocha C. A. Robie. J. Chromatogr, 511 : 177-183 (1990).

4. C. Cann-Moisan, J. Caroff, E. Girin. J. Chromatogr. 574 : 134-136 (1992).

Received: July 8, 1993
Accepted: September 9, 1993