High Performance Liquid Chromatographic Separation of Putrescine and Cadaverine using 2-Hydroxynaphthaldehyde as a Derivatizing Reagent

M.Y. KHUHAWAR, M.B. RIND AND M.A. QURESHI
Institute of Chemistry, University of Sindh, Jamshoro, Pakistan

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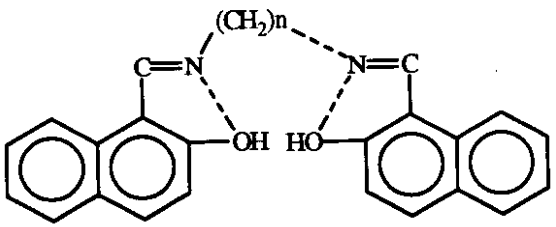
Summary: Biological active diamines, cadaverine and putrescine together with 1,3-propylenediamine and 1,2-propylenediamine react with 2-hydroxynaphthaldehyde to form bis (2-hydroxynaphthaldehyde) 1,5-diiminopentane (HN_2CA), bis(2-hydroxynaphthaldehyde) 1,4-diiminobutane (HN_2Pu) bis (2-hydroxynaphthaldehyde) 1,2-diiminopropane (HN_2Pn) and bis (2-hydroxynaphthaldehyde) 1,3-diiminopropane (HN_2PR). The derivatives are observed to be fluorescent in visible region. The wavelength of excitation (Ex) and emission (Em) were determined for optimal fluorescence of derivatives. The linear calibration for each of the diamine is observed at 0.32-1.6 μg/ml. The diamine derivatives HN_2Pu, HN_2Pn also separated on Nova Pak C-18 column when eluted with methanol. The spectrofluorimetric detection was obtained at 450 nm using Ex at 315 nm.

Introduction

The naturally occurring diamines 1,3-diaminopropane, 1,4-diaminobutane (Putrescine) 1,5-diaminopentane (Cadaverine) have been shown to be present ubiquitously in procarytotes and eucaryotes [1-4]. In mammals including man, putrescine is generally present in low concentration except in tissues that are growth stimulated or have a high cell proliferative compartment such as bone marrow [5]. Putrescine in mammalian cells is formed by direct decarboxylation of orinithine [1]. The interests in the determination of diamines have increased since Russell reported that patients with matastic cancer excreted increased levels of putrescine in urine [6]. A number of studies have been carried out to determine the concentration of diamines and polyamines in biological fluids and tissues, the variations in concentrations have been determined in normal, tumor affected tissues and after treatment with different anticancer agents. A number of different methods have also been reported for the determination of the diamines and polyamines, including spectrophotometry [7], spectrofluorimetry, thin layer chromatography [8], ion, exchange chromatography [9], gas chromatography [10] and liquid chromatography [11,12]. The liquid chromatographic procedure involves mostly minhydrin [8] o-phthaldehyde [13], 9-fluorenylmethyl chloroformate [14], N-succinimidyl-3-ferrocenylpropinate [15], benzoyl-chloride [16], 3,5-dinitrobenzoylchloride [17], densyl chloride [18], fluorescamine [19] acetyl-acetone [20] 8-hydroxyquinoline sulphonic acid [21] and 2-(1-pyrenyl)ethyl chloroformate [22] as reagents for post column and precolumn derivatization. Spectrophotometric, spectrofluorimetric or electrochemical detection is conveniently obtained.

Recently 2-hydroxy-1-naphthaldehyde (NH) has been used as a derivatizing reagent for putrescine (Pu), cadaverine (CA), 1,2-diaminopropane (pn) and 1,3-diaminopropane (PR) for HPLC separation and determination of the diamines [23]. Spectrophotometric detection was obtained at 260 nm. In the present work the diamine derivatives, HN_2Pu, HN_2CA, HN_2Pn and HN_2PR (Fig. 1) have been characterized on spectrofluorimetry and their HPLC separation has been examined using spectrofluorimetric detection.



(1) HN_2Pr n=3
(2) HN_2Pu, n=4
(3) HN_2CA, n=5

Fig. 1: Structural diagrams diamines derivatives.

Results and Discussion

The potentials of the diamine derivatives HN_2Pn, HN_2PR, HN_2Pu and HN_2CA, conveniently prepared by simple condensation were investigated for the fluorometric determination of diamines. The absorption spectrum of each of the derivative was recorded in methanol. Different wavelength of maximum absorbances were fixed as excitation wavelengths and emission wavelength was scanned. The wavelength at which maximum fluorescence intensity was observed was selected. Again the emission wavelength was fixed and excitation wavelength was scanned and optimized excitation wavelength was confirmed. The results of this study are summarized in Table-1. Linear calibrations were obtained with 0.32-1.6 µg/ml by plotting fluorescence intensity against concentration with coefficient of correlation (γ) of 0.999, 0.982, 0.9987 and 0.999 for HN_2Pn, HN_2PR, HN_2Pu, and HN_2CA respectively, HN_2Pu, has highest sensitivity followed by in sequence HN_2Pn, HN_2PR, HN_2CA (Fig. 2).

Table Optimized conditions for fluorometric determination of diamine derivatives

	Compound	Excitation wavelength	Emission wavelength
1.	Bis(2-hydroxy-1-naphthaldehyde-1,2-diiminopropane	240	350
2.	Bis(2-hydroxy-1-naphthaldehyde) 1,3-diiminopropane	247	450
3.	Bis(2-hydroxy-1-naphthaldehyde)-1,4-diiminobutane	310	460
4.	Bis(2-hydroxy-1-naphthaldehyde)-1,5-diiminopentane	395	450

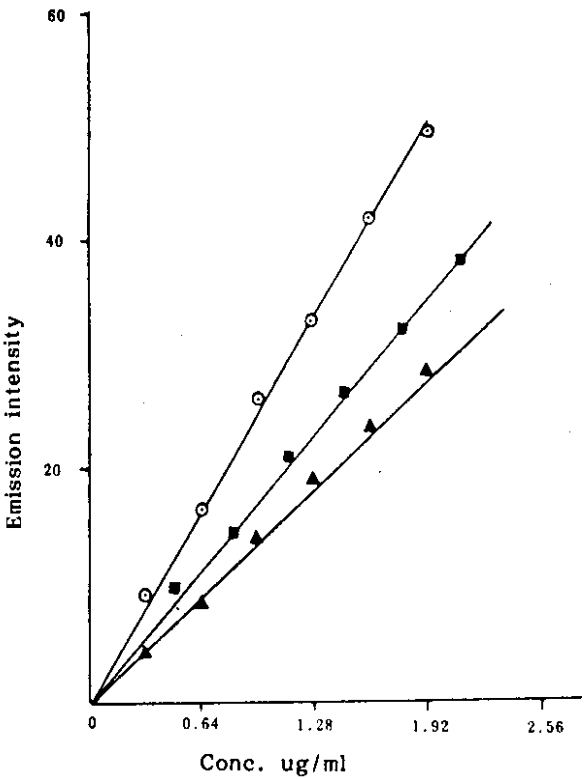


Fig. 2: Calibration plate of 1) HN_2Pu 2) HN_2PR and 3) HN_2CA

The diamine derivatives indicated a reasonable sensitivity to enable to determine the diamine at sub µg/ml, however the derivatizing reagent HN also indicates some fluorescence at the wavelengths selected for HN_2PR, HN_2Pn, HN_2Pu, and HN_2CA and would interfere when present in excess, added as derivatizing agent. It was therefore HPLC coupled with spectrofluorimetric detector was used for the separation of HN, HN_2PR, HN_2Pu and HN_2CA. All of the compounds were

easily eluted and separated from the reversed phase C-18 column when eluted with methanol, using flow rate of 0.9 mL/min. The detection was obtained at 450 nm (Em) using 315 nm as excitation wavelength (Fig. 3). However, HN_2Pn, coeluted with HN_2PR and failed to separate using the described conditions.

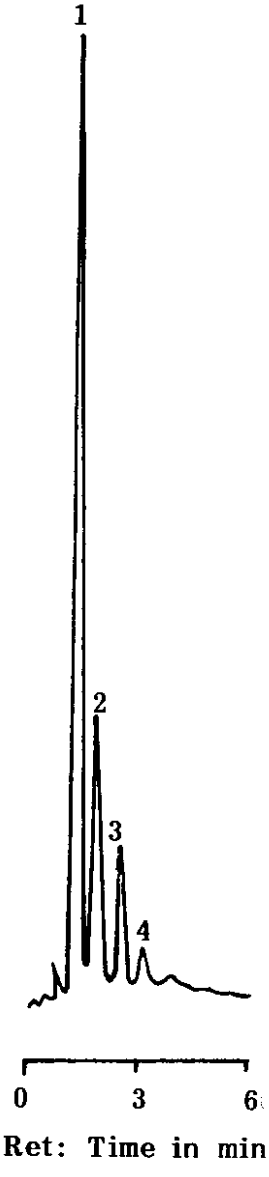


Fig. 3: HPLC separation of 1) NH 2) PR 3) Pu 4) CA as derivatives of NH.
Column Nova Pak C-18, 5μ (150 x 3.9 mm id). Elution with methanol 0.9 mL/min. Detection Spectrofluorometeric EX 315 nm, and Em 45 nm.

When the derivatization procedure was used HN completely separated form diamine derivatives and did not interfere with the determination of the diamines. Linear calibration curves for each of the PR, Pu and CA at the optimized wavelengths for the fluorimetric determination were obtained with 0.4-2.5 μg/mL, 2-6 μg/mL 0.8-6 μg/mL respectively. The detection limits measured at least three times the background noice were obtained 40 ng/mL,. 200 ng/mL, 80 ng/mL for PR, Pu and CA respectively.

Experimental

Bis(2-hydroxynaphthaldehyde) 1,2-diiminopropane (HN_2Pn), bis (2-hydroxynaphthaldehyde) 1,3-diiminopropane (HN_2PR), bis (2-hydroxynaphthaldehyde) 1,4-diiminobutane (HN_2Pu) and bis (2-hydroxynaphthaldehyde) 1,5-diiminopentane (HN_2CA) were prepared by condensation of HN with Pn, PR, Pu or CA in 2:1 molar ratio in ethanol as reported [23]. Fluoresence measurements were carried out on Hitachi F-1200 spectrofluorimeter. Absorption spectra of the compounds were recorded on Hitachi 220 spectrophotometer. Hitachi 655A liquid chromatograph connected with Hitachi F-1200 spectrofluorimeter, Rheodyne 7125 injector, and Hitachi 561 recorder was used. Separations were made on a Nova Pak C-18 (150 x 3.9 mm id) (Millipore Ltd. USA) column.

Derivatization procedure

Solution (1-5 mL) containing (PR, Pu, CA) diamines (0-150 μg) each was added sodium bicarbonate buffer pH 8 (2 ml) and HN (5 mL) (1 mg/ml in methanol). The mixture was warmed at 60°C for 5 min. and volume was adjusted to 25 mL with methanol. The solution (1 μl) was injected on HPLC column and solutes were eluted with methanol 0.9 mL/min and spectrofluorimetric detection was made at Ex:315 and EM: 450 mm.

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