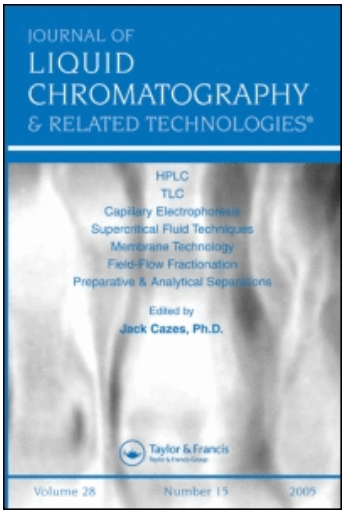


**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Selection of A Suitable Solvent System for the Separation and Quantitation of Histamine

Benny Washington[a]; Margaret O. Smith[a]; T. Joan Robinson[a]; Richard F. Ochillo[a]
[a] Laboratories of Pharmacology, Toxicology Biomedical Research Center Xavier University of Louisiana, New Orleans, Louisiana

**To cite this Article** Washington, Benny , Smith, Margaret O. , Robinson, T. Joan and Ochillo, Richard F.(1991) 'Selection of A Suitable Solvent System for the Separation and Quantitation of Histamine', Journal of Liquid Chromatography & Related Technologies, 14: 7, 1417 — 1424
**To link to this Article: DOI:** 10.1080/01483919108049329
**URL:** http://dx.doi.org/10.1080/01483919108049329

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(7), 1417-1424 (1991)

# SELECTION OF A SUITABLE SOLVENT SYSTEM FOR THE SEPARATION AND QUANTITATION OF HISTAMINE

**BENNY WASHINGTON, MARGARET O. SMITH, T. JOAN ROBINSON, AND RICHARD F. OCHILLO**
*Laboratories of Pharmacology and Toxicology*
*Biomedical Research Center*
*Xavier University of Louisiana*
*New Orleans, Louisiana 70125*



## ABSTRACT

The suitability of 79:21, 85:15 and, 90:10 of phosphate buffer: 50% methanol, as mobile phases, to separate and quantitate histamine in small plasma volume ($\leq$300 $\mu$l) was investigated by using a Dionex BioLC System coupled to a pulsed amperometric detector. Also, special attempts were made to reduce the time of analysis of each sample. Methylhistamine was used as an internal standard. The best results were obtained with the 79:21 mobile phase with concommittant reduction in analysis time from the current 1 sample per 90 min to 5 samples per 60 min suggesting that the method is suitable and cost effective.

## INTRODUCTION

Histamine is a common constituent of biological samples. Its pharmacodynamics in the body has not been

fully elucidated. Also, histamine has been implicated in the malfunction of a number of body functions including the immune, cardiovascular system and the gastrointestinal system. Consequently a number of drugs have been developed which are targeted to histamine and the body system at which its malfunction could lead to disease. In order to maximize the potential of developing new therapeutic agents targeted to malfunctions caused by histamine imbalance and also, to elucidate precisely the pharmacodynamics of histamine, it was necessary to develop a separation and a quantitative method for histamine which is rapid and cost effective. Therefore, there was a need to develop reliable and rapid methods for the determination of histamine in non-derivatized small plasma samples.



The high performance liquid chromatography (HPLC) has been used to measure histamine in plasma and the results of this attempt strongly indicated the significance of selecting the correct mobile phase [1-6]. Davis et al. [7] reported that histamine could be separated and quantitated by HPLC in derivatized samples with a 0.07 M sodium phosphate:methanol buffer. The problem with these HPLC methods was that the separation and quantitation of histamine is more than 90 minutes, which makes the method relatively expensive when large number of samples are involved.

In order to overcome this problem, we have modified the HPLC Method by selecting a suitable mobile phase and using a Dionex BioLC system to separate and quantitate histamine in small plasma volume in the shortest time ever reported.

## MATERIALS AND METHODS

### Reagents

Histamine dihydrochloride and methylhistamine were purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals were "HPLC grade" or reagent grade and were obtained from Fisher Scientific Instrumentation Laboratory, Atlanta, GA..

### Standard Preparation

Standards were prepared by dissolving 6 mg histamine dihydrochloride and 1 mg methylhistamine (internal standard) in 50 ml of a 0.12M phosphate buffer. They were aliquoted into 1.5ml microcentrifuge tubes stored frozen until use at a -80 degrees centigrade. The working solutions were prepared on the day of the experiment by diluting standards to working concentrations (0.03-0.2 pmols). These solutions were stored on ice until they were injected into the Dionex BioLC System for analysis.

### Plasma Sample Preparation for Histamine Analysis

Plasma samples for histamine analysis were prepared by adding 200 pmols of methylhistamine to 300

$\mu$l of blood sample in a 1.5 ml heparinized microcentrifuge tube. The sample was centrifuged (2000 rpm) for 20 min at 4°C. The plasma from these samples was removed and diluted with phosphate buffer to make a 1:1 dilution. This mixture was then vortexed for approximately 30 seconds and then heated in boiling water for 3-4 minutes to denature protein. The boiled mixture was centrifuged for 10 minutes to separate the protein residue from the sample. The supernatant which contained histamine was removed and stored on ice throughout the experiment.



**Instrumentation**

Detection of histamine was done using a Dionex BioLC, Instrument with a quaternary gradient pump and a pulsed amperometric detector. The detector selectively determines picomole levels of histamine. The potential voltage was set at +1.05 volts with an output range of 3-30 nA throughout the experiments. The basic chromatographic module contained a metal-free, high pressure injector. Histamine was separated through a stainless steel C-18 Zorbax ODS 4.5mm ID x 25cm (5 $\mu$m) column.

A phosphate buffer was prepared which consisted of 0.12M $NaH_2PO_4$, 0.1M NaOH and 5.6mg of Lauryl Sulfate. This buffer was used to prepare the appropriate mobile phase mixtures as follows: (1)

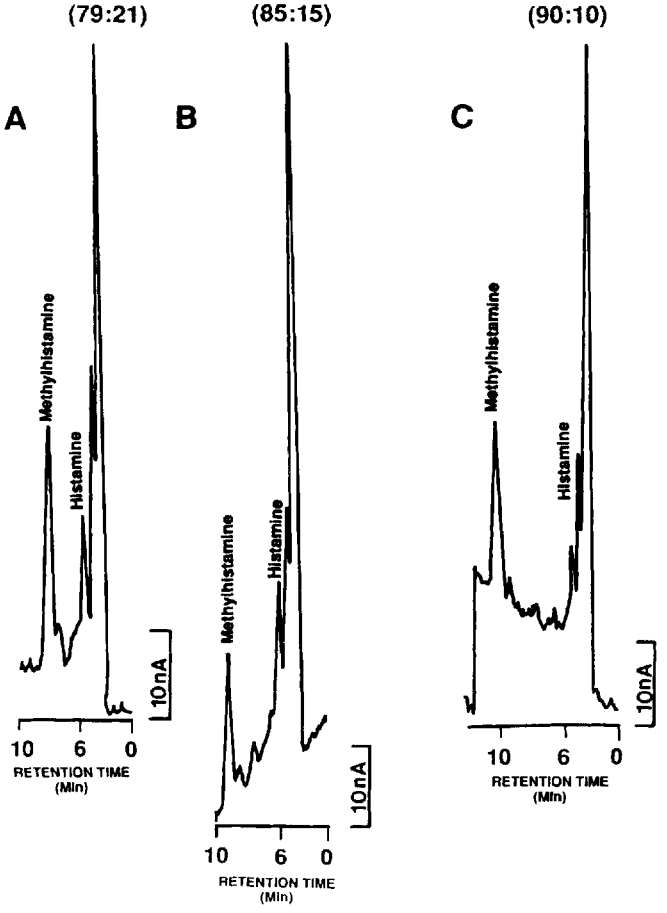


Figure 1. HPLC chromatogram of separating and quantitating histamine and methylhistamine in small plasma samples with (A) phosphate buffer-50% methanol (79:21),(B) phosphate buffer-50% methanol (85:15); and (c) phosphate buffer-50% methanol (90:10).

phosphate buffer-50% Methanol (79:21); (2) phosphate buffer-50% methanol (85:15) and(3) phosphate buffer-50% methanol (90:10). The pH of each solution was adjusted to 5.6 and filtered under vaccum through a 0.45 micron millipore filter.

## RESULTS AND DISCUSSION

The suitability of 3 different mobile phases for the separation and quantitation of histamine in as little as 300 $\mu$l of plasma were tested. The best results were obtained with the 79:21 of phosphate buffer: 50% methanol as the mobile phase (Fig. 1A). The use of this mobile phase provided excellent separation between histamine and methylhistamine. The retention time for histamine and methylhistamine were 6 and 10 minutes respectively. The limit of sensitivty for histamine was 0.03 pmols/ml of plasma and the recovery was 79.9%. When the mobile phases 85:15 and 90:10 (phosphate: 50% methanol) were used, histamine eluted close to the solvent front (Fig. 1B and 1C).

In conclusion we have described a suitable mobile phase for a rapid and accurate means for the separation of histamine and methylhistamine in non-derivatized small plasma samples. The use of this mobile phase reduces analysis time from the current 1

sample per 90 min to 5 samples per 60 min which suggest that it is cost effective, and if use with HPLC can provide a means for the quantitation of histamine in other biological samples.

**ACKNOWLEDGEMENT**

This work was supported by RCMI Grant #SRCR, 1-G12-RR050750 and #RR08008 from NIH. We also thank Miss Rita Sparks for typing this manuscript.



**REFERENCES**

1. Leroy, P., Nicolas, A., Moreau, A., Electrochemical detection of sympathomimetic drugs, following pre-column o-phthalaldehyde derivatization and reversed-phase high performance liquid chromatography, J. Chromatogr., 282:561-569, 1983.

2. Schubert, C.W., Andrew, R.W., Matson, W.R., Morrison, J., Zink, E.W. The HPLC analysis of subnanogram quantities of histamine in biological fluids using coulometric multielectrode electrchemical detection. In: Abstract, Pittsburgh Conference and Exposition on Anal. Chem. and Applied Spectrscropy, 1984:050.

3. Harsing, L.G., Nagashima, H., Duncalf, D. Sylvester Vizi, E., Goldiner, P.L., Determination of histamine concentrations in plasma by liquid chromatography/electrochemistry. Clin. Chem. 32 (10) 1823-1827, 1986.

4. Tsuruta, Y., Kohashi K., Ohkura, Y., Determination of histamine in plasma by high-speed liquid chromatography, J. Chromatography, 146:490-493, 1978.

5. Granerus, G. Wass, U., Urinary excretion of histamine, methylhistamine (1-MeHi) and methyllimidazoleacetic acid (MeImAA) in mastocytosis: comparison of new HPLC methods with other present methods, Agents Actions 14: 341-345, 1984.

6. Robert, J.C., Vatier, J., Nguyen Phuoc, B.K., Bonfils, S., Determination of histamine, methylhistamine and histamine-o-phthaldialdehyde complexes by two high-performance liquid chromatographic procedures, J. Chromatgr. 273: 275-287, 1983.

7. Davis, T.P., Gehrke, C.W., Gerke, C.W. Jr., High-performance liquid chromatographic separation and fluorescence measurement of biogenic amines in plasma, urine, and tissue, Clin. Chem. 24: 1317-1324, 1978.