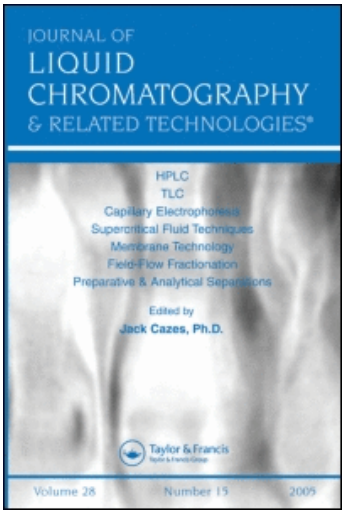

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Washington, Benny , Smith, Margaret O. , Robinson, T. Joan , Olubadewo, Joseph O. and Ochillo, Richard F.(1991) 'A Rapid Qualitative and Quantitative Method of Assaying Histamine in Small Plasma Volume', Journal of Liquid Chromatography & Related Technologies, 14: 11, 2189 — 2200
To link to this Article: DOI: 10.1080/01483919108049683
URL: http://dx.doi.org/10.1080/01483919108049683

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(11), 2189-2200 (1991)

A RAPID QUALITATIVE AND QUANTITATIVE METHOD OF ASSAYING HISTAMINE IN SMALL PLASMA VOLUME

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ABSTRACT

A modified method for a qualitative and quantitative determination of histamine in small plasma volume ($\leq$300 μl) was developed. According to this method, blood samples containing methylhistamine, the internal standard, are centrifuged to collect plasma. These plasma samples which contain underivatized histamine are injected into a Dionex BioLC System coupled with a pulsed amperometric detector. Histamine and methylhistamine are separated through a C-18 Zorbax ODS 4.5mm ID x 25cm (5 microns) column. Histamine is quantitated by comparing histamine peak height with that of known quantity of the internal standard. The sensitivity of the method is 0.03 pmols. The peak heights were found to be linearly related to histamine concentrations providing a quantitative means of assaying histamine in biological samples. The retention time of histamine was 6 min in contrast to that of methlyhistamine which was 10 min.

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Introduction

There have been numerous procedures reported for measuring histamine in biological samples. Of these, bioassay [1], fluorometry [2-6], fluorometry combined with HPLC [7], double or single-isotope radioenzymatic assays [8-10], immunoanalysis [11], gas chromatography-mass spectrometry [12,13], and electrochemical detector combine with HPLC [14,15]) have been the most widely used techniques. Most of these techniques, however, suffer from disadvantages such as low specificity and sensitivity, the possible appearance of contamination, and they are time consuming. The first reliable chemical determination of histamine in biological materials was a fluorometric assay based upon derivatization of histamine with o-phthaldehyde (OPT) [2]. Leroy et al. [14] reported that reacting some amines with OPT and 2-mercaptoethanol (2-MCE) yields condensation products that can be oxidized electrochemically at a moderate potential. Harsing et al. [15] derivatized histamine with OPT, and they were able to detect as little as 0.45 pmols of standard histamine using HPLC-electrochemical detector (HPLC-EC). Although, Harsing et al. [15] reported detecting as little as 0.45 pmols of histamine, the method used to detect this amount

required as much as 4 ml of plasma. Morel and Delaage [11] reported acylation of histamine with N-hydroxysuccinimide and succinyl-glycinamide (NHS-SGA) converting histamine into a highly immunoreactive derivative. This immunoreactive derivative can be detected with a monoclonal antibody in as little as 100 μl of plasma. Problems that are associated with this method: (1) the antibody not only has an affinity for the acylated histamine but also has affinity for other metabolite in the samples, and (2) the reproducibility of the reagent to acylate histamine in biological samples is low and the procedure is laborous. Since our investigation involved the rat as an experimental animal model, and only small volume of plasma can be obtained from a rat repetitively, it was necessary for us to develop a rapid and highly sensitive procedure for quantitating histamine in small plasma volume.

In this communication, we describe a rapid qualitative and quantitative assay for underivatized histamine in small plasma volume. This rapid assay was developed by using a Dionex BioLC System coupled with a pulsed amperometric detector. Compared to the methods mentioned above, including the one by Morel and Delaage [11], our method does not only requires

as little as 300 μl of plasma for the quantitation of histamine, but also, it is rapid and more sensitive. The method can be used for a qualitative and quantitative analysis of histamine with methylhistamine as the internal standard.

Methods and Materials

Reagents

Histamine dihydrochloride and methylhistamine were purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals were "HPLC grade" or reagent grade.

Standard Preparation

Histamine dihydrochloride standards were prepared by dissolving 6 mg in 50 ml of a 0.12M phosphate buffer and aliquoted into 1.5ml microcentrifuge tubes. They were stored frozen at -80 degrees centigrade for future use. The working solution was 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 injected into the HPLC.

Plasma Sample Preparation for Histamine Analysis

Plasma samples for histamine analysis were prepared by adding 200 pmols of methylhistamine, the

internal standard, to 300-400 μl of blood sample in a 1.5ml heparinized microcentrifuge tube. The sample was centrifuged (2000 rpm) for 20 min at 4°C. The plasma from this sample was removed and diluted with phosphate buffer to make a 1:1 dilution. This mixture was then vortexed for approximately 30 seconds. After vortexing, the sample was heated in boiling water for 3-4 minutes to denature protein in the plasma. The boiled mixture was centrifuged for 10 minutes to separate the protein residue from the sample. The supernatant from the preparation was removed and stored on ice until injected into the HPLC.

Instrumentation

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

The analytical mobile phase was a phosphate buffer which consisted of 0.12M NaH_2PO_4, 0.1M NaOH, 19 μM of Lauryl Sulfate and methanol (79:21, v/v). The pH

was adjusted to 5.6. This solution was filtered under vaccum through a 0.45 micron millipore filter prior to use. The details of the selection of a suitable solvent system for the separation and quantatation of histamine is being published separately [16].

Standard Curve

Known amounts of histamine (225-900 pmols) and methylhistamine were added to 300 μl of plasma. These samples were prepared according to the procedure described above. To construct the standard curve, 10 μl portions of each standard was injected into the HPLC system. The actual amounts of each histamine standard used ranged from 0.03 to 0.2 pmols/10 μl, while the amount of methylhistamine, the internal standard, was held constant at 1.58 pmols/10 μl.

Results And Discussion

The results from identifying and quantitating histamine in as little as 300 μl of plasma are presented in Fig. 1. The retention time for histamine and methylhistamine were 6 mins and 10 mins, respectively. The most suitable mobile phase used to separate and quantitate histamine was a mobile phase of (v/v) 79:21% (phosphate buffer:methanol).

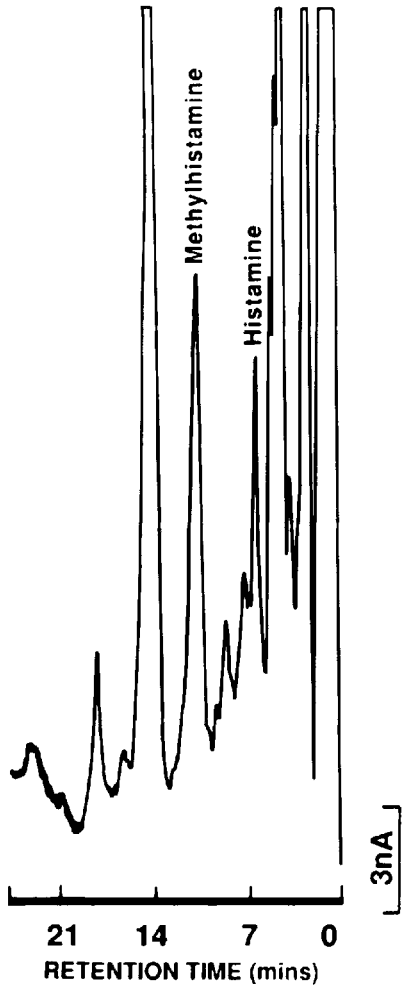

Figure 1. Chromatogram of histamine and methylhistamine (1.05 and 1.58 pmols respectively). The chromatographic conditions are the same as those mentioned in the text.

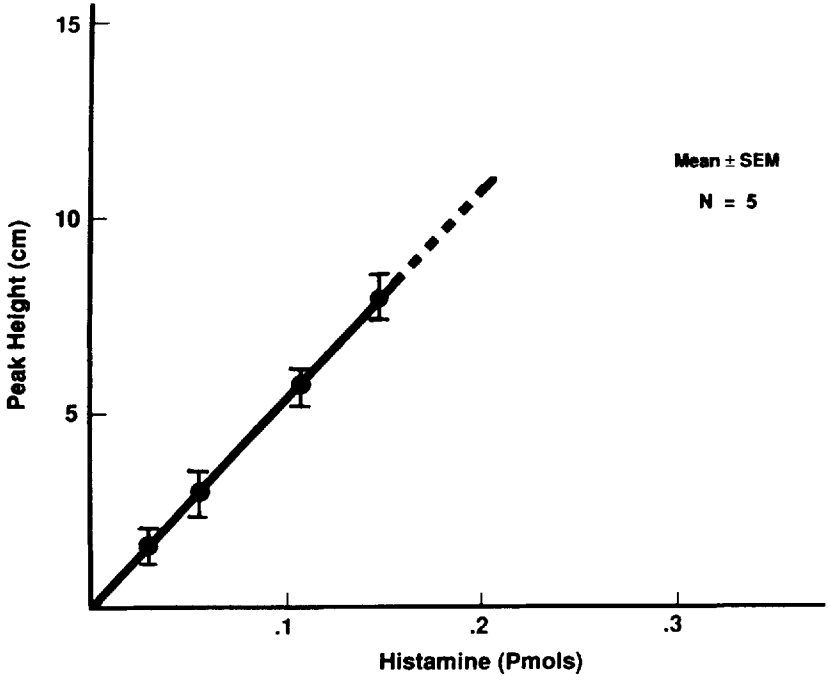

Figure 2. Standard curve for histamine. The peak height of histamine were linearly related to histamine concentration. Each point represents the mean ± S.E.M. of 5 determinations.

Therefore, we decided to use this mobile phase in this investigation.

The peak heights were linearly related to histamine concentrations which provided a standard curve for histamine in the samples (Fig. 2). The correlation coefficient was excellent (r=0.99). The sensitivity of detection was 0.03 pmols and the recovery of histamine was 79.9%.

The first reliable chemical determination of histamine in biological materials was a fluorometric assay [2]. Since then, other procedures have been developed but they all lacked specificity and sensitivity, and are time consuming. A previous report for the determination of histamine in human plasma was based upon deproteination of plasma, purification, precolumn off-line derivatization, and subsequently injecting the sample into the HPLC [14]. Our method eliminates the purification and off-line derivatization steps.

This modified method is a simple method which has several advantages: (1) it requires as little as 300 μl of plasma to quantitate histamine relative to the fluorometric method which requires 4 ml of plasma [14]; (2) the sensitivity for the detection of histamine is less than 0.03 pmols which is less than what has been previously reported [11,15]; and (3) it is rapid. The method is suitable for qualitative and quantitative analysis of histamine. We have successfully applied the method to determine histamine in biological samples in some of our series of investigations [17-19].

ACKNOWLEDGEMENT

This investigation was supported by RCMI Grant #SRCR, 1G12RR050750 from NIH. Thanks to Miss Rita

Sparks for typing this manuscript and Kinh Nguyen for his laboratory assistance.

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