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Ion-pair extraction of histamine from biological fluids and tissues for its determination by high-performance liquid chromatography with fluorescence detection

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ABSTRACT

A procedure for the selective ion-pair extraction of histamine from various biological fluids and tissues by using the ion-pairing agent bis(2-ethylhexyl)hydrogenphosphoric acid has been developed. This procedure enabled routine determination of histamine in plasma, urine and tissue samples of humans and rats by reversed-phase high-performance liquid chromatography with fluorescence detection using *o*-phthaldialdehyde.

INTRODUCTION

High-performance liquid chromatography (HPLC), especially when combined with fluorescence detection using *o*-phthaldialdehyde (OPA) as the fluorogenic reagent, is among the most sensitive and frequently used methods to quantitate histamine in various biological fluids and tissues [1–13]. Some of these methods allow the determination of histamine and other biogenic amines in some biological fluids without the need of an extraction step prior to HPLC. However, these techniques lack specificity with respect to histamine and are therefore unsuitable for the rapid and routine determination of histamine in various matrices. When rapid analysis is desired, histamine has to be selectively extracted from plasma and other biological fluids and tissues prior to the HPLC analysis. *n*-Butanol extraction and other extraction procedures have been found very useful [9,10,12–17] but not completely free from interferences by histamine-like substances [10].

This paper describes a highly selective ion-pair extraction of histamine from plasma, urine and stomach samples of humans and various tissues of rats. It uses the ion-pairing agent bis(2-ethylhexyl)phosphoric acid (B2EHPA) for the determination of histamine by HPLC with fluorescence detection.

EXPERIMENTAL

Chemicals and reagents

L-Histamine dihydrochloride, L-norepinephrine, *n*-heptane, methanol and acetonitrile of HPLC quality, and OPA were from Fluka (Neu-Ulm, Germany). B2EHPA and dopamine were from Serva (Heidelberg, Germany). Putrescine, spermidine, 1-methylhistamine, tyramine, serotine and octopamine were bought from Sigma (Munich, Germany).

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HPLC conditions

The HPLC system used consisted of an LKB Model 2156 microprocessor-controlled pump with titanium capillaries (Bromma, Sweden). Samples were loaded with a Rheodyne high-pressure sampling injection valve provided with a 200-μl sample loop. The column (250 mm × 4 mm I.D.) was packed with Nucleosil C_6H_5 (7 μm particle size) from Macherey-Nagel (Düren, Germany). For the determination of histamine in plasma, urine and tissue, a gradient elution between solvent A (25 m*M* sodium dihydrogenphosphate) and solvent B (25 m*M* sodium dihydrogenphosphate in acetonitrile, 30:1, v/v) was used. The flow-rate was 1.0 ml/min. The column effluent was monitored with a Model RF-530 variable-wavelength Shimadzu fluorimeter (Kyoto, Japan) equipped with a 10-μl cell. The wavelengths were set at 450 nm for emission and 350 nm for excitation. Chromatograms and all calculations were performed on a Model C-R3A Shimadzu integrator. Ion-pair extraction of histamine was performed on a vortex mixer (Hobein, Switzerland). Exactly the same derivatization procedure with OPA was used as previously described [10].

Sample preparation and ion-pair extraction procedure

Blood was taken and plasma prepared exactly as described elsewhere [10]. Plasma was used without pre-treatment. Urine samples were diluted (1:1, v/v) with 25 m*M* potassium phosphate buffer (pH 7.4). Biopsy specimens were taken from corpus mucosal of healthy persons (n = 12). Whole tissues from humans and from Lewis rats (200–300 g) were homogenized at 0°C in 25 m*M* potassium phosphate buffer (pH 7.4) using various volumes depending on the histamine content of the tissue. Plasma, urine and tissue suspensions were frozen and stored at −80°C until use. A 2-ml aliquot of plasma, urine, tissue suspension or a stock solution of histamine in 25 m*M* potassium phosphate buffer (pH 7.4) were vortex-mixed for 5 min with 6 ml of a daily freshly prepared 50 m*M* B2EHPA solution in n-heptane in conical 12-ml glass tubes, and the mixture was then centrifuged (4000 g, 20 min). A 5.5-ml aliquot of the supernatant was taken up and vortex-mixed for 2 min with 400 μl of 0.1 *M* HCl in order to re-extract histamine into the aqueous solution. This mixture was centrifuged (4000 g, 5 min) and a 200-μl aliquot of the aqueous acidic phase was derivatized following alkanization as described [10].

Recoveries were determined from the slopes of calibrations curves, which were generated by separate addition (triplicate) of various amounts of histamine to the matrices. The relative standard deviation (R.S.D.) was determined from simultaneous ion-pair extraction of histamine from six identical samples of each matrix.

RESULTS

Fig. 1. shows a chromatogram of a mixture of standards of histamine and spermidine in 0.01 *M* HCl solution. The retention times (t_R) for spermidine and histamine were 5.03 ± 0.06 and 7.03 ± 0.05 min (mean ± S.D., n = 20), respectively. From these analyses the concentration of histamine was determined to be 10.9 ± 0.4 ng/ml

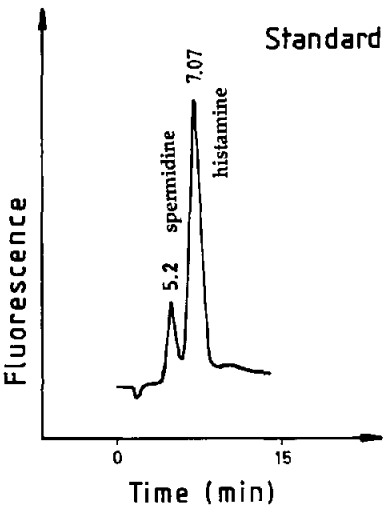

Fig. 1. Chromatogram of a mixture of standards of spermidine (1000 n*M*) and histamine (100 n*M*) in 0.01 *M* HCl solution as their OPA adducts. The following gradient elution between solvent A (25 m*M* NaH_2PO_4) and solvent B (25 m*M* NaH_2PO_4–acetonitrile, 30:1, v/v) was used: 2 min at 50% B, 0.8 min linear to 100% B, and 7.2 min at 100% B. The flow-rate was 1.0 ml/min.

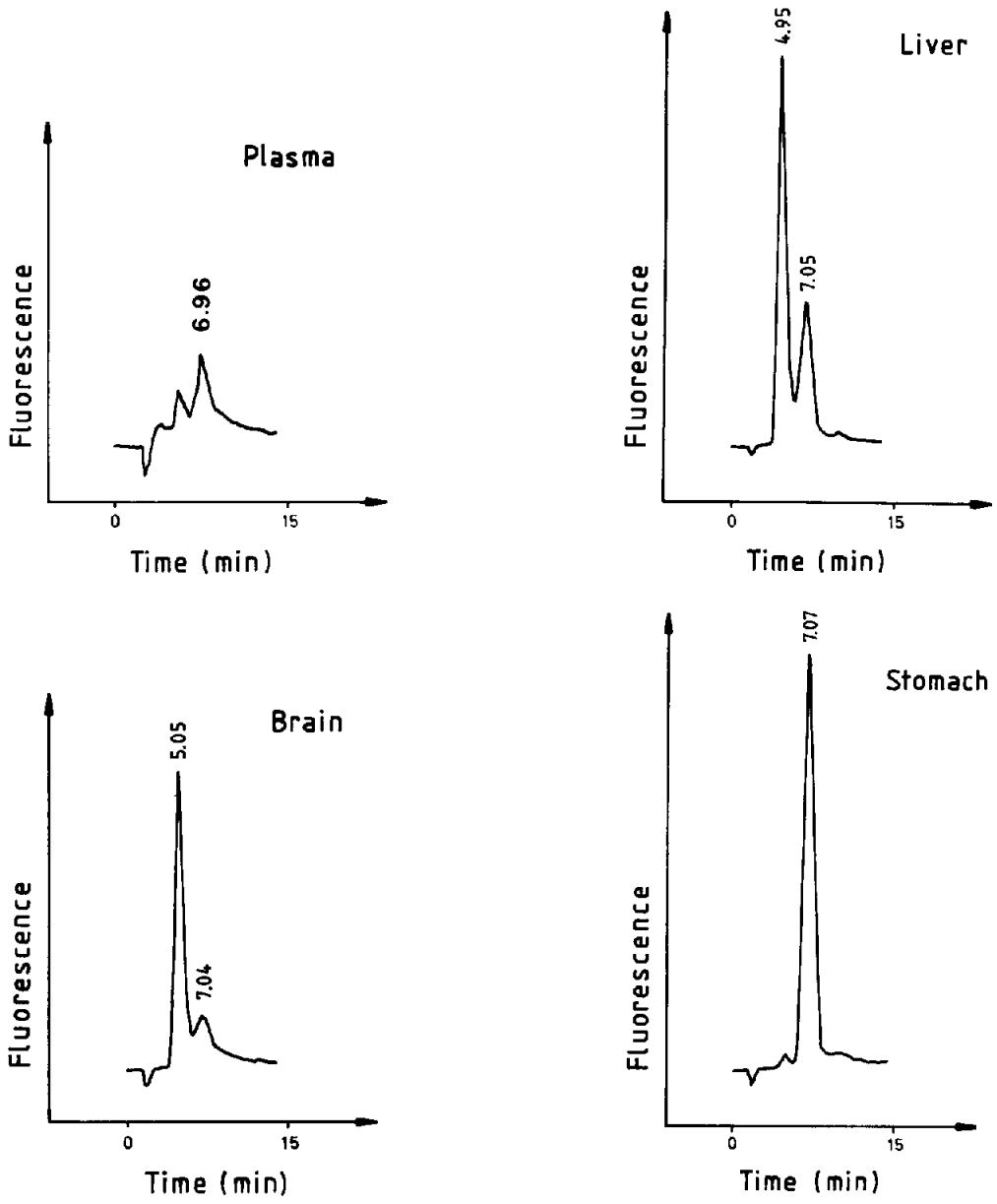

Fig. 2. Chromatograms of histamine in human plasma, rat liver tissue, rat brain, and rat stomach, after ion-pair extraction with B2EHPA as described in Experimental. Gradient elution as in Fig. 1.

(mean ± S.D.) with a coefficient of variation (C.V.) of 1.8. Fig. 2 shows chromatograms of histamine in human plasma and in suspensions of rat liver, rat brain and rat stomach after ion-pair extraction with B2EHPA. The chromatograms show two major peaks, which were found to correspond to spermidine (5.1 min) and histamine (7.1 min).

Table I gives the recoveries for the ion-pair extraction of histamine from 25 m*M* phosphate buffer (pH 7.4), plasma, urine and tissue suspensions. The coefficients of correlation between histamine found and histamine added were higher than 0.984 for all matrices. Despite low recovery values, the detection limit of the method (0.09 ng/ml plasma) is satisfactory for the routine de-

TABLE I

OVERALL RECOVERIES OF HISTAMINE FROM PLASMA AND TISSUE SUSPENSIONS

Ion-pair extraction with B2EHPA was performed under optimum conditions. For dilution, 25 m*M* potassium phosphate buffer (pH 7.4) was used.

Source	Recovery (mean ± R.S.D.) (%)	Dilution (v/v)
25 m*M* K^+ phosphate buffer (pH 7.4)	57 ± 6	No
Plasma	49 ± 7	No
Urine	51 ± 6	1:1
Liver	49 ± 6	1:50
Stomach	50 ± 6	1:500
Brain	47 ± 8	1:8

TABLE II

HISTAMINE CONCENTRATIONS IN PLASMA AND URINE OF HEALTHY PERSONS AND HISTAMINE CONTENT IN STOMACH TISSUE OF HUMANS AND TISSUES OF LEWIS RATS

Histamine concentrations and contents were determined using the recoveries of Table I.

Source	Concentration or content of histamine, (mean ± S.D., $n = 6$)	
Human		
Plasma	0.48 ± 0.2	ng/ml
Urine	14.5 ± 8.3	ng/ml
Stomach[a]	286 ± 50	nmol/g tissue
Rat		
Liver	11.7 ± 3.6	nmol/g tissue
Stomach	455 ± 86	nmol/g tissue
Brain	1.30 ± 0.23	nmol/g tissue

[a] $n = 12$.

termination of histamine, even in human plasma.

When histamine solutions in 25 m*M* potassium phosphate (pH 7.4) were used, histamine was quantitatively extracted into the ion-pairing solution using B2EHPA concentrations higher than 50 m*M*. Molarities of the potassium phosphate buffer higher than 25 m*M* decreased the extraction recovery of histamine, most probably because of competition of K^+ with the histamine cation. On the other hand, the best recovery rate of histamine from the organic phase into the hydrochloric acid solution or other aqueous solutions, such as potassium phosphate buffer, sodium hydroxide and sodium chloride, was only 57% (Table I). This finding may explain the similar recovery values of histamine from aqueous buffered solutions, plasma, urine and tissue suspensions.

The histamine contents in plasma and urine samples of humans, in stomach biopsy specimens of humans and in various rat tissues were determined (Table II). Our data lie within the same range as those reported by other investigators [1–4,6,7,14–16,18]. The spermidine content of brain and liver tissue, but not that of stomach tissue, was much higher than the content of histamine. The ratio of spermidine to histamine in brain tissue was determined to be of the order of 600:1. Similar ratios have been reported in the literature [4,19]. Despite the high sperimidine content of brain and liver tissues, the HPLC system used separates sufficiently the OPA adducts of histamine and spermidine, and thus allows accurate determination of histamine even in these tissues within 15 min.

DISCUSSION

B2EHPA has been reported to be selective for some metal and organic cations [20,21]. This study demonstrates the utility of this ion-pairing agent for the highly selective extraction of histamine from various biological fluids and tissues of humans and rats. The ion-pair extraction procedure described here permits the routine and sensitive determination of histamine in various matrices by HPLC and fluorescence detection (Fig. 2) using OPA, one of most widely used detection techniques for histamine [1–13]. Except for spermidine, no other biogenic amines listed in *Chemicals and reagents* and no amino acids were found to be extracted by this procedure. The satisfactory HPLC separation of histamine from spermidine and the *ca.* 40-fold lower fluorescence intensity of the spermidine–OPA adduct (Fig. 1)

allow accurate determination of histamine, even in the presence of high amounts of spermidine in rat liver and rat brain tissue (Fig. 2; Table II).

The ion-pair extraction of histamine by B2EHPA is more specific than the widely used extraction of histamine by *n*-butanol. The latter requires a high pH, at which not only histamine but all biogenic amines exist as undissociated molecules. On the contrary, ion-pair extraction of histamine by B2EHPA can be performed at a physiological pH, at which histamine exists almost entirely as a singly protonated molecular cation. In previous reports we have shown that ranitidine may disturb the determination of histamine in plasma when *n*-butanol extraction is used [10,22]. In this study ranitidine was shown not to interfere with histamine.

CONCLUSION

The ion-pair extraction described here is characterized by high selectivity, accuracy and reproducibility. The overall recovery of the method is relatively low, but it is sufficient for the accurate quantitation of histamine in matrices of low histamine content by HPLC. The extraction procedure may also be useful in other detection techniques, such as radioimmunoassay and gas chromatography–mass spectrometry.

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