High-Performance Liquid Chromatographic Determination of Plasma Histamine after Pre-Column Derivatisation with o-Phthaldialdehyde

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Summary
A reversed-phase high-performance liquid chromatographic (HPLC) method has been developed for the sensitive and highly selective determination of histamine in plasma. This method includes selective extraction of histamine from plasma, pre-column derivatisation in aqueous phase with o-phthaldialdehyde (OPA) and HPLC analysis. The fluorescence of the histamine-OPA-complex was monitored at wavelengths of 350nm excitation and 460nm emission, after isocratic elution with a mixture of 0.2M NaCl and methanol. The reproducibility of this method including extraction, derivatisation and detection of histamine was >95% in a range of 0.35–17.6pmol. The HPLC precision was 99 ± 1% at 4pmol of histamine. The lower limit of detection was 88fmol. A significantly increased concentration of plasma histamine was detected in patients (n = 46) with various liver diseases (0.3–5.2ng/ml). In comparison the plasma histamine levels of healthy blood donors were in the range of 0.0–0.4ng/ml (p < 0.01).

Key Words
High-performance liquid chromatography
Pre-column derivatisation
o-Phthaldialdehyde
Plasma histamine determination
Liver disease

Introduction
Selective and sensitive determination of histamine in human plasma is difficult mainly because of many interfering substances. Different analytical methods have been described for the determination of histamine in plasma using fluorimetric detection. These include batch reactions [1] as well as HPLC methods using pre-column derivatisation [2–7], and post-column derivisation [8]. The lower limit of the detection of histamine achieved by these methods ranged from 2.2 down to 0.1pmol for pre-column derivatisation and down to 0.05pmol for post-column derivatisation.

According to the present literature plasma levels of histamine in healthy persons are described to be below 1ng/ml [1, 9]. Histamine determination in plasma is not accurate with batch reactions [1, 10], because interfering or auto-fluorescing substances from the plasma cannot be separated. The disadvantage of the very accurate post-column derivatisation method is the complicated and expensive instrumentation. Other HPLC methods do not detect histamine in concentrations below 0.2ng/ml. The HPLC method is ideal for separating histamine from interfering substances which are autofluorescent or condensate with o-phthaldialdehyde. The present paper describes a rapid HPLC method for the quantitative and sensitive determination of histamine in plasma which can be used in healthy humans and in patients with liver diseases. This method is based on a three-step butanol extraction of histamine from the protein-free plasma supernatant, with subsequent pre-column derivatisation with o-phthaldialdehyde and HPLC analysis on a Nucleosil 5 C18 analytical column under isocratic eluation conditions.

Experimental
Chemicals
L-histamine dihydrochloride, L-aspartate, L-glutamic acid, L-methionine, L-phenylalanine, L-tryptophane, and DL-octopamine hydrochloride were obtained from Serva (Heidelberg, FRG). Glycine, L-alanine, L-cysteine, L-histidine and L-tyrosine were from Merck (Darmstadt, FRG) and d-phthaldialdehyde was from Fluka (Buchs, Switzerland). All chemicals were of the highest available purity.

Chromatographic Apparatus and Conditions
The HPLC system consisted of a Model 2156 (LKB) microprocessor-controlled HPLC pump with titanium capillaries. Samples were loaded with a Rheodyne high-pressure sampling injection valve provided with a 200μl sample loop. The column (250mm x 4mm i.d.) was packed with a reversed-phase material, Nucleosil C18 5μm (Macherey-Nagel, Düren, FRG). Column effluent was monitored with a Model RF-530 (Shimadzu, Japan) variable wavelength

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fluorescent detector. The cell volume was 12µl. The wavelengths were set to emission 450nm and to excitation 350nm. Chromatogram recordings and all calculations were performed on a Model C-R3A integrator (Shimadzu, Japan).

The mobile phase was a 5:95 mixture of (A) methanol and (B) a 45:55 mixture of 0.2N NaCl solution and methanol, adjusted to pH 3.0 with 1N HCl. The flow rate was 0.5ml/min.

Special Precautions

The glass tubes were very carefully cleaned by the following procedure: three times with tap water, once with destilled water and twice with twice-distilled water. Twice-distilled water was used for the preparation of all solutions. The o-phthaldialdehyde reagent solution was prepared daily and was stable for 12h in darkness at 0°C.

Plasma Preparation

All blood samples were taken between 7.00 and 8.00 A.M. from a cubital vein. Volunteers and patients had fasted and lain in bed for at least 10h. The first 5ml of blood were not used, because histamine may be liberated at the injection site [1]. 10ml of blood were withdrawn with a Lithium-heparine-monovette (Sarstedt) and put in an ice-water bath after gentle mixing. Within 30min the blood was centrifuged for 15min at 1000g and 2°C (SM-24 rotor, Sorvall; DU Pont). 4ml of the obtained plasma were mixed with 2ml of 2M HClO₄ and centrifuged of 10min at 1800g and 2°C. The supernatant was decanted and stored at −28°C until used for analysis.

Fresh frozen plasma was mixed with 2M HClO₄ (2:1) and centrifuged for 10min at 1800g and 2°C (GSA rotor). To 200ml of the supernatant 1ml of a histamine solution (200ng/ml) was added and frozen in small samples at −28°C. This pool was used to calibrate the HPLC histamine peak.

HPLC Analysis of Plasma

A 1.0ml aliquot of the sample was pipetted into a 10ml glass tube, and 0.6ml of 5N NaOH, 0.8g NaCl crystals and 5.0ml n-butanol were added to it. The tube was closed with a glass stopper, shaken with a “head-over-head” shaker for 20min and centrifuged for 5min at 1500g. 4.7ml from the organic phase were transfered to a glass tube containing 3.0ml of 1N NaCl-saturated NaOH. This mixture was shaken and centrifuged in the same manner. 0.5ml of 0.1n HCl and 4.0ml heptane were added to 4.0ml of the organic phase. This mixture was again shaken for 20min and centrifuged as described above. 0.2ml of the acidic aqueous phase was made alkaline with 40µl 1N NaOH. For derivatisation 5µl 0.05 wt-% OPA in methanol (OPA-reagent) were added to it. This derivatisation mixture was shaken for 45sec and the reaction was stopped after exactly 4min with 20µl 3N HCl.

Results and Discussion

The volume of the OPA-reagent (OPA-R) needed for the derivatisation of histamine was optimized, because the OPA concentrations suggested in the literature [1] showed high underground noise. For the determination of the optimal concentration various OPA-R volumes (5–200µl) were added to 1ml of aqueous histamine solution (1mg/ml) and 0.2ml 1N NaOH. Fig. 1 shows the development of fluorescence intensity as a function of the amount of OPA-R added for the derivatisation of histamine. The optimal OPA-R volume was found as 25µl for 1ml of histamine solution (= 5µl OPA-R for 200µl histamine solution). At the optimum OPA-R volume of 5µl a linear relationship was found between the fluorescence intensity and histamine concentration, ranging from 0.17 to 88.0pmol.

The time for the complete development of the histamine-OPA-complex was found to be 4min (Fig. 2). Therefore this time was accurately maintained for all investigations, although variation from 3–6min can be accepted, as can be seen from Fig. 2.

Fig. 3 presents the stability of the histamine-OPA-complex. These results were obtained under optimized conditions of the optimum OPA-R volume and the optimum reaction time (4min). The histamine-OPA-complex was very stable up to 5h after derivatisation. An autosampler with a programming derivatisation device would be useful for serial histamine determination.

HPLC determination of histamine in plasma of patients in hepatic coma showed an unknown peak (Fig. 4). This peak could be traced down as ranitidine [10]. This drug is routinely administered to patients in severe liver diseases because H₂-receptor antagonists have proven to be beneficial for stress ulcer prophylaxis in liver disease [11].