Determination of Histamine and Leukotrienes from Basophils in Cell Supernatants by High-Performance Liquid Chromatography

Dirk Beil, Heiko Kinder, Angelika Paschke, and **Hans Steinhart**
Institute of Biochemistry and Food Chemistry, Department of Food Chemistry, University of Hamburg, 20146 Hamburg, Germany

Dieter Vieluf
Department of Dermatology and Allergy, University Hospital Hamburg-Eppendorf, 20246 Hamburg, Germany

Heidrun Behrendt
Department of Experimental Dermatology and Allergology, University Hospital Hamburg-Eppendorf, 20246 Hamburg, Germany

Johannes Ring
Department of Dermatology am Biederstein, Technical University Munich, 80802 München, Germany

Abstract

A reversed-phase high-performance liquid chromatographic method with gradient elution for the separation of the mediator substances histamine and the leukotrienes C_4 (LTC_4), D_4 (LTD_4), and E_4 (LTE_4) is described. The detection occurs fluorimetrically after automated precolumn derivatization with o-phthaldialdehyde. All components are chromatographically separable. Because of the different excitation and emission wavelengths, only the most important biological active mediators histamine and LTC_4 are determinable in one parallel chromatographic run. The method is examined by linearity and precision tests and is applicable to biological sample matrices like cell supernatants of human basophils enriched by Percoll-density gradient centrifugation and stimulated for mediator release by anti-IgE. The established method is suitable to separate the mediators from other matrix components. The determination limit for histamine is 55.0 µg/L and that for LTC_4 16.0 µg/L, referring to the reference solutions. Therefore, a fast, economical method for the common determination of the most important mediators histamine and LTC_4 is established. This method is also suitable for high sample amounts in routine medical analysis.

Introduction

Apart from the mast cells, basophil granulocytes are substantially involved in the manifestation of clinical symptoms of allergic reactions. Among the mediators released by basophils, histamine and the leukotrienes A_4 (LTA_4), C_4 (LTC_4), D_4(LTD_4), and E_4(LTE_4) are of great importance. To our knowledge, no method for the common but specific determination of these mediators has been devised. In routine medical analysis, the determination of histamine often occurs fluorimetrically with the use of an autoanalyzer. This method has the advantage of ensuring an extensive and fast sample throughput but captures all compounds able to react with *o*-phthaldialdehyde (OPA) and therefore leads to unspecific results (1,2). As an alternative, the expensive enzyme-linked immunosorbent assay (ELISA) is used (3) for the histamine determination as well as for the total leukotriene determination (4). The determination of biogenic amines such as histamine by reversed-phase high-performance liquid chromatography (HPLC) is a frequently used method. The separation usually involves a C_8 or C_{18} column with a gradient elution and a mixture of methanol and inorganic buffer. The detection is often fluorimetrically performed after OPA precolumn derivatization (5–7). The analysis of leukotrienes by reversed-phase HPLC is extensively referred to in the literature (8–10). For example, Powell described chromatographic determination on a C_{18} phase with an eluent consisting of an aqueous trifluoroacetic acid solution and acetonitrile. Ultraviolet (UV) detection was photometrically performed at 280 nm (8).

It is commonly known that primary amino functions react with OPA, so it was predicted that all leukotrienes with primary amino functions would react likewise. The leukotrienes LTC_4, LTD_4, and LTE_4 have primary amino functions, but LTA_4 does not. So it should be possible to determine these derivatives fluorimetrically after OPA precolumn derivatization and chromatographic separation by HPLC. From anti-IgE stimulated human basophils enriched by Percoll-density gradient centrifugation, a method for the common and specific determination of the most important mediators such as histamine, LTC_4, LTD_4, and LTE_4 was established using the HPLC technique.

Experimental

Chemicals and reagents

The phosphate buffer solution contained 6.90 g sodium dihydrogen phosphate (Merck, Darmstadt, Germany), 0.19 g ethylenediaminetetraacetic acid, tetrasodium salt (Sigma, Deisen-

hofen, Germany), and double-distilled water; the solution was 1000 mL, and had a pH level of 3.1. Gradient-grade methanol was obtained from Merck. A complete OPA reagent solution was obtained from Sigma, as were the histamine, LTC_4, LTD_4, and LTE_4.

Reference solutions

The concentrations of histamine were 55.5, 83.3, 111.0, 222.0, 388.5, 555.0, and 1110.0 µg/L (in phosphate buffer solution–methanol [70:30, v/v] mixture). The concentrations of leukotrienes were 16.7, 33.3, 166.3, 332.5, and 666.5 µg/L each (in phosphate buffer solution–methanol [70:30, v/v]). The reference solutions were used for determining the compounds' retention times and for testing the linearity and precision of the method.

Equipment

The chromatographic system consisted of the following components: an HPLC Pump 64 (ERC, Alteglofsheim, Germany), a #21564 dynamic mixing chamber (ERC), a gradient controller model 300 benchtop (Autochrom, Milford, MI), a #3312 degasser (ERC), Promis II autosampler enabled for precolumn derivatization (Spark, Emmen, Netherlands), an RP-8 Spherisorb column (250 × 4-mm i.d., 5-µm particles) (Ziemer, Mannheim, Germany), an RF 535 fluorescence detector (Shimadzu, Kyoto, Japan), a D-2000 integrator (Merck-Hitachi, Darmstadt, Germany), a minor model compressor (Jun-Air, Nørresundby, Denmark), and a type 125 column oven (ERC). An FP 550 fluorimeter was obtained from Jasco (Gross-Umstadt, Germany), and Minisart RC 15 disposable syringe filters (0.45 µm) were obtained from Sartorius (Göttingen, Germany).

HPLC method

The reaction of the mediators with a complete OPA solution was automatically performed in an autosampler provided with a mixing chamber and was completed in 2 min. The same volumes of sample and reagent solution were used. The separation of the indole derivatives occurred by low-pressure gradient elution with a phosphate buffer solution–methanol mixture. Eluent A was a phosphate buffer solution–methanol mixture (70:30, v/v), and eluent B was methanol. The injection volume was 20 µL (sample loop), and the temperature was 25°C.

The time program of linear gradient elution is given in Table I. Fluorimetric HPLC detection was performed at fixed excitation (340 nm) and emission (455 nm) wavelengths. Previously the excitation and emission wavelengths of the OPA derivatives of histamine, LTC_4, LTD_4, and LTE_4 were determined by a fluorimeter.

Enrichment of basophils and stimulation

In order to test the HPLC method for biological sample matrices, the cell supernatants of human basophils enriched by Percoll-density gradient centrifugation and stimulated for mediator release by anti-IgE were measured (11).

Results and Discussion

The fluorimetrically determined maximum excitation and emission wavelengths are shown in Table II. It is obvious that the determined maximum excitation and emission wavelengths of each OPA derivative were very different.

An automated wavelength change of the fluorescence detector could not be performed. For the common detection of the different derivatives in one parallel chromatographic run, it was necessary to select fixed excitation and emission wavelengths. The measurement was performed at wavelengths that were as near as possible to the fluorimetrically determined excitation and emission maxima to ensure sufficient detection sensitivity.

The examination shows that a chromatographic separation of all compounds, in particular histamine, LTC_4, LTD_4, and LTE_4, was possible. The retention times are given in Table III.

The biologically active and most important mediators, histamine and LTC_4, were detectable with sufficient sensitivity at the same fixed excitation and emission wavelengths of 340 and 455 nm, respectively, in contrast to LTD_4 and LTE_4. Therefore,

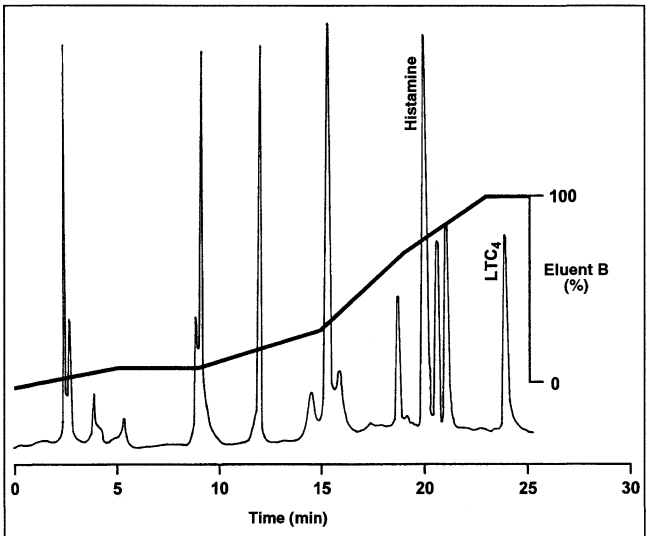

Figure 1. HPLC chromatogram of histamine (t_R, 20.4 min) and LTC_4 (t_R, 24.2 min) from anti-IgE stimulated basophils after OPA precolumn derivatization. Separation on a reversed-phase C_8 column (Spherisorb 5 µm, 250 × 4-mm i.d.) with a methanol gradient system (eluent A, phosphate buffer solution–methanol [70:30, v/v]; eluent B, methanol) at 25°C. Fluorimetric detection was at 340 nm (excitation wavelength) and 455 nm (emission wavelength).

Table I. Time Program of HPLC Linear Gradient Elution for the Determination of Histamine, LTC_4, LTD_4, and LTE_4

Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)	Elution mode
0	100	0	0.9	initial
5	90	10	0.9	linear
9	90	10	0.9	plateau
15	70	30	0.7	linear
19	30	70	0.7	linear
23	0	100	0.7	linear
35	100	0	0.7	linear

Table II. Fluorimetrically Determined Maximum Excitation and Emission Wavelengths of Histamine, LTC_4, LTD_4, and LTE_4

Compound	OPA derivative	
	Maximum excitation wavelength (nm)	Maximum emission wavelength (nm)
Histamine	360	441
LTC_4	338	455
LTD_4	337	475
LTE_4	341	465

Table III. Retention Times of Mediators Determined by HPLC

Compound	Retention time (min)
Histamine	20.4
LTC_4	24.2
LTD_4	32.4
LTE_4	37.6

these wavelengths were selected for further investigations, and the analysis of LTD_4 and LTE_4 was accorded less importance.

The calibration functions for the reference solutions of histamine and LTC_4 were calculated, and the linearities were tested. The correlation coefficient for histamine was 0.9998 and that for LTC_4 was 0.9996. The measurement precisions were in any case between the range of 2.0 and 6.5% (10 replicates each).

The method developed is also suitable for biological sample matrices. Figure 1 shows an HPLC chromatogram of the mediators released from human basophils stimulated by anti-IgE. The identity of the detected mediators was tested by standard addition. Mediator concentrations calculated on the basis of this chromatogram were 500 µg/L for histamine and 50 µg/L for LTC4, referring to the reference solutions. Determination limits of 55.0 and 16.0 µg/L for histamine and LTC_4, respectively, were calculated. The appearance of OPA derivatives of accompanying substances (amino acids, peptides, and blood proteins) is conspicuous but does not interfere with the evaluation under the selected chromatographic conditions.

Because the cleanup of the mediators from biological sample components is not necessary, this method facilitates a fast, economical mediator analysis with an extensive sample throughput and represents an interesting alternative to the conventional methods in routine medical analysis.

Acknowledgments

The authors wish to thank Dr. B. Hitzfeld for her scientific assistance and Mrs. D. Gerding for her technical support.

References

1. U. Rohr, W. König, and F. Selenka. Einfluß von Pestiziden auf die Freisetzung von Histamin, chemotaktischen Faktoren und Leukotrienen aus Rattenmastzellen und menschlichen Basophilen. *Zbl. Bakt. Hyg.* **181:** 469–86 (1985).
2. T.J. Schröder and L.R. Hanrahan. Purification of human basophils using mouse monoclonal IgE. *J. Immunol. Methods* **133:** 269–77 (1990).
3. P. McBride, D. Bradley, and M. Kaliner. Evaluation of a radioimmunoassay for histamine measurement in biological fluids. *J. Allergy Clin. Immunol.* **82:** 638–46 (1988).
4. S. Höxtermann, T. Auer, and P. Altmeyer. Zelluläre In vitro-Diagnostik mittels CAST-ELISA. *Allergologie* **7:** 287–91 (1995).
5. M. Roth. Fluorescence reaction for amino acids. *Anal. Chem.* **43:** 880–82 (1971).
6. K. Petridis and H. Steinhart. Automatische Vorsäulenderivatisierung mit o-Phthaldialdehyd (OPA). *Z. Lebensm. Unters. Forsch.* **201:** 256–60 (1995).
7. K. Hermann, G. Frank, and J. Ring. High performance liquid chromatography for the separation of histamine, its precursor, and metabolites: Application to biological samples. *J. Liq. Chromatogr.* **18:** 189–204 (1995).
8. W.S. Powell. Reversed-phase high-pressure liquid chromatography of arachidonic acid metabolites formed by cyclooxygenase and lipoxygenases. *Anal. Biochem.* **148:** 56–69 (1985).
9. M. Köller, W. Schönfeld, J. Knöller, K.D. Bremm, and W. König. The metabolism of leukotrienes in blood plasma studied by high-performance liquid chromatography. *Biochem. Biophys. Acta* **833:** 128–34 (1985).
10. M. Dawson and C.M. McGee. Comparison between ethanol and methanol for the reversed-phase high-performance liquid chromatographic analysis of leukotriene B_4 and its metabolites in cell incubation supernatants. *J. Chromatogr.* **532:** 379–86 (1990).
11. J. Ring, D. Beil, K. Beauregard, I. Vieluf, D. Vieluf, H. Kinder, H. Behrendt, and H. Steinhart. Xenobiotische Nahrungsmittelbestandteile und Nahrungsmittel-Allergie: In vivo- und in vitro-Untersuchungen bei Patienten mit Apfelallergie. In 5. Statuskolloquium des PUG am 5. und 6. März 1996 im Forschungszentrum Karlsruhe. F. Horsch, P. Friebel, A. Seidel, and A. Wiebel, Eds. Projekt Umwelt und Gesundheit (PUG), *Forschungszentrum Karlsruhe*: 247–59 (1996).

Manuscript accepted February 6, 1998.