www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Influence of sample condition on the histamine detecting capability of reversed-phase ion-pairing HPLC

Ebrahimnejad H., *Gheisari H.R. and Nazer A. H.K.

Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Abstract

Histamine is an autacoid that exerts multiple functions in physiologic and pathophysiological processes. Histamine analysis in biological samples or certain high risk food is of a great importance for disease diagnosis or monitoring the quality of food products. The aim of this study was to evaluate the effect of sample condition on histamine detecting capability of reversed-phase ion-pairing HPLC, a reliable and less difficult method comparing some other HPLC techniques. Histamine detectability was evaluated in a buffer system relevant to the food and bio-samples; under pH 5.0, 7.0 and 9.0 while regulating temperature at 25°C, 37°C and 100°C. The results showed that acidifying the sample, perhaps due to development of more positive charged histamine, provides a better resolution either at 25°C or 37°C ($P<0.05$). The study suggested that maximum ionization of histamine fulfils the requirements of ion-pair RF-HPLC to act as a potent and selective technique in histamine separation and quantification.

Keywords: Histamine; ion-pair reversed-phase HPLC; resolution

To cite this article: Ebrahimnejad H, HR Gheisari and AHK Nazer, 2013. Influence of sample condition on the histamine detecting capability of reversed-phase ion-pairing HPLC. Res. Opin. Anim. Vet. Sci., 3(9), 293-296.

Introduction

Histamine, a known mediator of several biological reactions, is regarded as one of the most important multifunctional biogenic amines in biomedicine. This low molecular weight amine can exclusively be synthesized by α-decarboxylation of L-histidine *via* L-histidine decarboxylase (HDC, E.C. 4.1.1.22) reaction. This sole enzymatic reaction takes place in various cells throughout the body (Dy and Schneider, 2004; Parsons and Ganellin, 2006) and also pathogens like Gram-positive and Gram-negative bacteria (Gallagher et al., 1989).

Possibly due to the chemical properties of histamine, that allow it to be versatile in binding to targets, pleiotropic responses can be elicited by histamine in the human body. Furthermore, it has a potent role in hematopoiesis, neurotransmission, immunomodulation, circadian rhythm, wound healing and the regulation of cell proliferation and angiogenesis (Maintz and Novak, 2007). Histamine production during processing and/or storage of certain foods, usually by the action of spoilage bacteria, might be associated with food-borne poisoning (Lawley et al., 2008).

The multiple biological actions of histamine dictate the need of a proper and efficient histamine-determining method in different biological samples and in various high risk foods such as scombroid fish, Swiss cheese, Spanish-style green olive, certain sausages etc. (Jay et al., 2005).

Among various methods available for histamine determination of samples, high performance liquid chromatography (HPLC) offers a precise method of histamine analysis because of its sensitivity and wide range of linearity (Hwang et al., 1997). Since, there is no any chromophoric and fluorophoric moiety in the structure of histamine, it can be detected *via* HPLC by a number of derivatization steps which are sometimes cumbersome (Oguri and Yoneya, 2002).

Intact histamine can be separated and quantified by ion-pair chromatography with reversed-phase columns whilst ion-pair reagents, added to the mobile phase, enhance separation efficiency and reduce the risk of peak tailing (Vind et al., 1991; Toyo'oka, 2008).

Corresponding author: Gheisari H. R., Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Not surprisingly, the acquired molecular properties of histamine in individual environments might have an impact on the ion-pairs formation between charged histamine and ion-pair reagents and subsequently affect the resolution of reversed-phase high performance liquid chromatography (RP-HPLC). The objective of the present study was to assess histamine detecting capability of RP-HPLC in different thermal and pH conditions. These conditions are relevant to food industry or biological samples and believably affect histamine specifications and its behavior. Hence, ambient temperature in food plants (25°C), normal human body temperature and thermal process in food industry (100°C; 30 min) were evaluated in histamine detecting capability of RP-HPLC. Furthermore, acidic, neutral and alkaline conditions, which are perceivable in different foods or bio-samples, were taken into consideration for histamine detectability of the procedure.

Materials and Methods

Preparation of thermal and pH conditions relevant to the food and biological samples

In order to evaluate the influence of sample conditions on histamine detecting capability of ion-pair RF-HPLC; histamine dihydrochloride solutions (H7250; Sigma-Aldrich Chemical Co.) with the concentration of 200 ppm were prepared in the potassium phosphate (KPi) buffers (0.02 M) under acidic, neutral and basic conditions (pH 5.0 and pH 7.0 and 9.0) and three temperatures (25°C, 37°C and 100°C) in a triplicate manner. Histamine content of the samples was quantitated *via* ion-pair RF-HPLC through 30 minutes. The precision of the method was determined by calculating the relative standard deviation (R.S.D., %) for the repeated measurements. Moreover, limit of detection (LOD) and limit of quantification (LOQ) were also evaluated to assess the validation and analytical sensitivity of the procedure.

Ion-pair RF-HPLC evaluation of histamine

Histamine detection by reversed-phase ion-pairing HPLC was performed as described previously (Cinquina et al., 2004) that does not require laborious pre-treatment, clean up and derivatization. All of the HPLC evaluations were done in triplicate.

Ion-pair RF-HPLC chemicals: HPLC-grade methanol (34869) and acetonitrile (34998), histamine dihydrochloride (H7250), 1,1-dimethylbiguanide hydrochloride (D150959) and 1-decanesulfonic acid sodium salt (30633) were supplied by Sigma-Aldrich (Munich, Germany). Analytical grade reagents (KH_2PO_4, $K_2HPO_4{\cdot}3H_2O$ and $HClO_4$) were supplied by Merck (Darmstadt, Germany). The water was obtained using the Milli-Q purification system of Millipore.

Ion-pair RF-HPLC solutions and eluents: The 1000 ppm stock solutions of 1,1-dimethylbiguanide hydrochloride and histamine dihydrochloride were prepared by dissolving them in HCl (0.1 M). Histamine working solutions at the concentration of 2, 5, 8, 10, 15, 30 and 50 mg L^{-1} in HCl (0.1 M) were prepared fresh every day. The final concentration of 1,1-dimethylbiguanide hydrochloride (chromatographic reference standard) added to all the solutions was about 5 mg L^{-1}. A mobile phase of buffer solution: methanol: acetonitrile (75:10:15, v/v/v) was delivered at a flow rate of 1.0 mL min^{-1} to the HPLC column. The buffer solution, 1.72 g of KH_2PO_4, 2.7 g of $K_2HPO_4{\cdot}3H_2O$ and 0.49 g of $C_{10}H_{21}O_3SNa$ (sodium 1-decanesulfonate) in 1 L water with a final pH of 6.7, was filtered through a 0.45-µm Millipore membrane filter, and degased before use in an ultrasonic bath (Sonorex Super® RK 100/H). The buffer solution was prepared at the time of use.

RF-HPLC equipment and conditions: The HPLC analyses were carried out using an Agilent HPLC system (Agilent 1100 series, Waldbronn, Germany) equipped with a quat pump G1311A, auto-sampler G1313A, degasser G1379A and a variable wavelength detector (VWD) G1314A. The separations were performed under isocratic conditions using a 5 µm C18 column of 250 mm×4.6 mm i.d. (Spherical, Optimal® ODS-H, Capital HPLC, UK) fitted with a 5 µm C18 guard column of 20 mm×4.6 mm (Spherical, Optimal® ODS-H, Capital HPLC, UK). The flow rate was 1.0 mL min^{-1}, the injection volume 30 µL and the VWD was positioned at a wavelength of 214 nm.

Statistical analysis

Values in all the experiments are represented as mean ± SD of experiments done in triplicate. Histamine detecting capability for each treatment *via* ion-pair RF-HPLC was considered significant at $P<0.05$ using paired t-test. Moreover, One-Way ANOVA was used to statistically analyze the differences between different treatments ($P<0.05$) followed by the Duncan's post-hoc test (Statistical package SPSS version 16.0)

Results

In this ion-pair RP-HPLC procedure, limit of detection (LOD) as the smallest possible concentration to deduce the presence of histamine was 1 ppm. Furthermore, limit of quantification (LOQ), the smallest histamine content that can be quantified with a specified degree of accuracy ($n = 10$), was 2 ppm.

In the buffered system, ion-pair RP-HPLC can quantify histamine at pH 5.0, 25ºC (Fig. 1) without a significant divergence from its initial level ($P<0.05$). At 37ºC, the deviation of histamine detection was 3.9% and 10.1% in pH 5.0 and pH 7.0, respectively.

Table 1: Detected histamine levels of potassium phosphate buffer solutions (0.02 M) regarding of unlike conditions (values in the same row followed by a different letter indicate a significant difference, P<0.05).

Treatment	Histamine (ppm, mean ± *SD*)		R.S.D.[1] (%)
	Actual level	Detected level	
pH 5.0, 25ºC	200 ± 0[a]	196.32 ± 1.77[a]	0.90
pH 5.0, 37ºC	200 ± 0[a]	192.20 ± 1.19[b]	0.62
pH 5.0, 100ºC	200 ± 0[a]	159.39 ± 2.80[b]	1.76
pH 7.0, 25ºC	200 ± 0[a]	183.42 ± 1.13[b]	0.62
pH 7.0, 37ºC	200 ± 0[a]	179.80 ± 1.75[b]	0.98
pH 7.0, 100ºC	200 ± 0[a]	157.10 ± 0.94[b]	0.60
pH 9.0, 25ºC	200 ± 0[a]	98.79 ± 1.29[b]	1.30
pH 9.0, 37ºC	200 ± 0[a]	107.67 ± 3.89[b]	3.61
pH 9.0, 100ºC	200 ± 0[a]	86.67 ± 7.68[b]	8.87

1 Relative standard deviation (RSD% = (SD/mean) × 100).

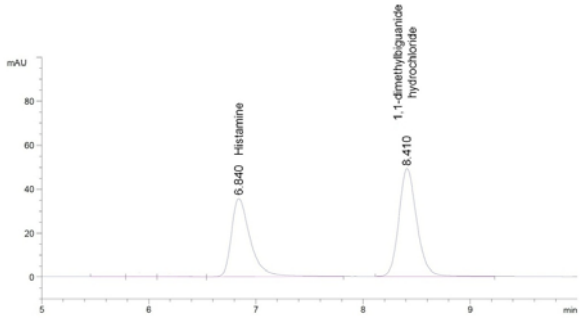

Fig. 1: HPLC chromatogram of histamine and 1,1-dimethylbiguanide internal standard in KPi buffer; conditions: column Optimal ODS-H C18 (250 mm×4.6 mm i.d.) at +35°C, buffer solution: methanol: acetonitrile (75:10:15, v/v/v) at a flow-rate of 1.0 mL min^{-1}, injection loop 30 µL and monitored at 214 nm.

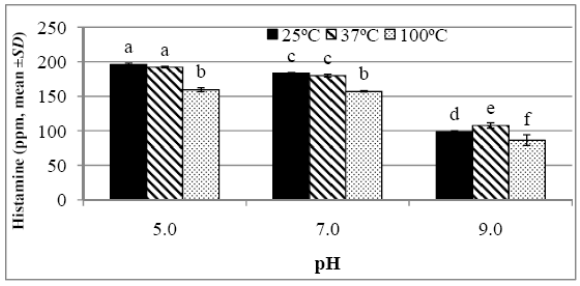

Fig. 2: Histamine quantification capability of ion-pair RF-HPLC in different thermal and pH conditions. (Different letters indicate a significant difference, P<0.05).

Intriguingly, quantitative analysis of histamine, *via* this set of ion-pair RF-HPLC, at 100ºC demonstrates a significant reduction after 30 minutes. Groups attributed to pH 9.0 exhibited a dramatic loss in histamine quantification from 46.16% to 56.66% (Table 1).

Concerning the actual level of histamine, KPi buffer of pH 5.0 at 37ºC reduced the histamine exploration point by ion-pair RF-HPLC (P<0.05), but this detection level does not show a significant difference with the condition of pH 5.0, 25ºC.

Warming up the samples to 100ºC for 30 minutes did not delineate a significant difference in histamine quantification level either in pH 5.0 or pH 7.0 (P<0.05).

Notably, the ion-pair RF-HPLC presented an identical quantification concept for 25ºC and 37ºC at pH 7.0; nonetheless, their histamine detection level was in conflict with other groups (Fig. 2).

Despite the drastic diminution of histamine recognition at pH 9.0 by this method, it could depict a distinguishable detection pattern between 25ºC, 37ºC and 100ºC (P<0.05).

Discussion

Primary aliphatic amine (pK_{a1} 9.4) and imidazole (pK_{a2} 5.8) are the two basic structural moieties of histamine. These groups make the monocation with different tautomers; the preferred form at physiologic pH value (96%) with a minor dicationic fraction (3%) and a very small amount of the neutral form (Cooper et al., 1990).

Ion-pair chromatography, as a type of RP-HPLC, has unravelled the limitations of regular RP-HPLC for poor peak-shape formation of basic compounds such as biogenic amines. The properties of ion-pair reagent, which permits the separation of charged analytes, and its concentration plays an important role in the selectivity of the method. Accordingly, efficient ion-pair formation, which might be affected by sample conditions, has a major impact on HPLC resolution.

In this study, histamine quantification capability of ion-pair RF-HPLC was sensibly affected by changing the pH of the sample. In the circumstances, basic compounds, like histamine, might sustain ionization while exposing to acidic environments. The more charge analyte develops, the better ion-pairs form.

Plausibly, at pH 5.0, histamine possesses more positive charge than at pH 7.0, due to its pK_a values, which results in a better resolution. Consistently, most of the histamine extraction solutions that have been used for the examination of high risk foods such as tuna fish, exhibit an acidic nature (Frattini and Lionetti, 1998; Cinquina et al., 2004; Innocente et al., 2007). It is likely that at pH 9.0 the majority of histamine molecules turns into neutral conformations which lessen the rate of ion-pair formation and finally leads to an inapplicable resolution.

Although histamine detection level at 100ºC during 30 min was lower than its actual level, but this

diminution in both pH 5.0 and pH 7.0 doesn't seem to be controversial with histamine thermostability phenomenon (Huss et al., 2004).

Finally, despite the findings regarding of histamine stability at low temperatures (Pratter et al., 1985; Marshik et al., 1999), temperature changes of the KPi buffer in the range of 25ºC to 37ºC didn't drastically affect histamine resolution. Hence, 35ºC was applied for the mobile phase perhaps due to proper equilibrium of ion-pair with the C18 column.

In conclusion, maximum ionization of histamine, before ion-pairing, is an inevitable process which can be conveniently achieved by lowering the pH. Ambient temperature (25ºC) inclined to the human body temperature has no histamine degradation effect and might create a better condition for histamine ionization and fast equilibration of ion-pairs with the stationary phase. Accordingly, ion-pair RP-HPLC could be a selective and powerful technique in histamine separation and quantification.

Acknowledgement

This study was supported financially by the Shiraz Univ. Research Council. We would also like to thank Dr M.J. Khoshnood for his technical advices.

References

Cinquina, A.L., Longo, F., Cali, A., De Santis, L., Baccelliere, R. and Cozzani, R. 2004. Validation and comparison of analytical methods for the determination of histamine in tuna fish samples. *Journal of Chromatography A*, 1032: 79-85.

Cooper, D.G., Young, R.C., Durant, G.J. and Ganellin, C.R. 1990. Histamine receptors, in: Hansch, C. and Emmett, J.C. (editors), Comprehensive Medicinal Chemistry. Pergamon, Oxford, pp: 323-421.

Dy, M. and Schneider, E. 2004. Histamine-cytokine connection in immunity and hematopoiesis. *Cytokine & Growth Factor Reviews*, 15: 393-410.

Frattini, V. and Lionetti, C. 1998. Histamine and histidine determination in tuna fish samples using high-performance liquid chromatography: Derivatization with o-phthalaldehyde and fluorescence detection or UV detection of "free" species. *Journal of Chromatography A*, 809: 241-245.

Gallagher, T., Snell, E.E. and Hackert, M.L. 1989. Pyruvoyl-dependent histidine decarboxylase. Active site structure and mechanistic analysis. *Journal of Biological Chemistry*, 264: 12737-12743.

Huss, L.L., Ababouch, L. and Gram, L. 2004. Assessment and Management of Seafood Safety and Quality. FAO, Rome.

Hwang, D.F., Chang, S.H., Shiua, C.Y. and Chai, T. 1997. High-performance liquid chromatographic determination of biogenic amines in fish implicated in food poisoning. *Journal of Chromatography B: Biomedical Sciences and Applications*, 693: 23-29.

Innocente, N., Biasutti, M., Padovese, M. and Moret, S. 2007. Determination of biogenic amines in cheese using HPLC technique and direct derivatization of acid extract. *Food Chemistry*, 101: 1285-1289.

Jay, J.M., Loessner, M.J. and Golden, D.A. 2005. Modern Food Microbiology. 7th edition. Springer, New York.

Lawley, R., Curtis, L. and Davis, J. 2008. The food safety hazard guidebook. The Royal Society of Chemistry, Cambridge, UK.

Maintz, L. and Novak, N. 2007. Histamine and histamine intolerance. *The American Journal of Clinical Nutrition*, 85: 1185-1196.

Marshik, P., Moghaddam, S., Tebbett, I. and Hendeles, L. 1999. Degradation of histamine solutions used for bronchoprovocation. *Chest*, 115: 194-199.

Oguri, S. and Yoneya, Y. 2002. Assay and biological relevance of endogenous histamine and its metabolites: application of microseparation techniques. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 781: 165-179.

Parsons, M.E. and Ganellin, C.R. 2006. Histamine and its receptors. *British Journal of Pharmacology*, 147 Suppl 1: S127-135.

Pratter, M.R., Marwaha, R.K., Irwin, R.S., Johnson, B.F. and Curley, F.J. 1985. Stability of stored histamine diphosphate solutions. Clinically useful information. *American Review of Respiratory Disease*, 132: 1130-1131.

Toyo'oka, T. 2008. Separation assay of histamine and its metabolites in biological specimens. *Biomedical Chromatography*, 22: 919-930.

Vind, S., Soondergaard, I., Poulsen, L.K., Svendsen, U.G. and Weeke, B. 1991. Comparison of methods for intestinal histamine application: histamine in enterosoluble capsules or via a duodeno-jenunal tube. Influence of fast and histamine-restrictive diet. *Allergy*, 46: 191-195.