

Status: Currently Official on 12-Feb-2025
 Official Date: Official as of 01-May-2022
 Document Type: General Chapter
 DocId: GUID-6F14AF8A-6F25-4FBD-AF0B-70FBC33A16FB_2_en-US
 DOI: https://doi.org/10.31003/USPNF_M13935_02_01
 DOI Ref: xep7y

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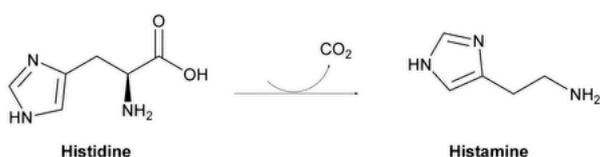
Add the following:

^<426> HISTAMINE TEST METHOD

INTRODUCTION

High levels of histamine in drug products can lead to severe adverse drug reactions, including flushing, itching, hives, shortness of breath, decreased blood pressure, increased heart rate, and even fatality.

A potential source of histamine impurity is peptone of animal origin, particularly fish peptone, which is a critical raw material used as a protein source in the fermentation process. Free histidine is a naturally-occurring amino acid present in animals that can be converted to histamine by histidine decarboxylase.



Fish peptone has a higher histamine contamination risk than other animal-derived peptones because some species of fish have larger quantities of histidine. Special attention must be paid to histamine levels when peptones of animal origin are used in the manufacturing process. This general chapter is intended to provide users with validated analytical methods to quantify the histamine levels in antibiotics.

Applicable requirements for histamine content are determined by the appropriate regulatory agency.

This chapter contains an LC-MS method to determine the histamine content. This method is validated for use with gentamicin sulfate, and must be validated for use with other drug substances.

HISTAMINE CONTENT IN GENTAMICIN SULFATE

• PROCEDURE

Solution A: [Acetonitrile](#)

Solution B: 10 mM [ammonium formate](#) in 0.1% [formic acid](#)

Sensitivity solution: 2 ng/mL solution of [USP Histamine Dihydrochloride RS](#)

Standard solutions: Accurately weigh an appropriate amount of [USP Histamine Dihydrochloride RS](#), and dissolve and dilute with *Solution B*.

Dilute further with *Solution B* to obtain a series of standard solutions (NLT 3) appropriately spaced and at concentrations appropriate for the assay within the range from 2 to 20 ng/mL.

Sample solution: Accurately weigh a suitable quantity of gentamicin sulfate, and dissolve and dilute with *Solution B* to prepare a 1 mg/mL solution.

Check standard: Accurately weigh an appropriate amount of [USP Histamine Dihydrochloride RS](#), and dissolve and dilute with *Solution B*.

Dilute further with *Solution B* to obtain a check standard within the range of the concentrations prepared for the *Standard curve*.

Mobile phase gradient: See [Table 1](#).

Table 1

Time (min)	Solution A (% v/v)	Solution B (% v/v)
0	90	10
4	40	60
5	40	60
5.01	90	10
7	90	10

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: MS

MS conditions

Ionization mode: Electrospray (+) ionization

Single-ion monitoring (SIM) mode: m/z 112.1

Probe temperature: 600°

Column: 2.1-mm × 10-cm; 1.7-µm packing [L68](#)

Column temperature: 35°

Flow rate: 0.5 mL/min

Injection volume: 1 µL

[NOTE—The approximate retention time of the histamine peak is 2.8 min.]

Standard curve

Samples: *Standard solutions*

Inject 1 µL of a series of *Standard solutions* and record the chromatograms and peak area of MS signals. Using the linear regression method, plot the histamine peak areas of the *Standard solutions* versus the histamine concentrations, and determine the standard curve best fitting the plotted points.

System suitability

Samples: *Sensitivity solution, Standard solutions, and Check standard*

Suitability requirements

Linearity: The correlation coefficient (*R*) of the *Standard curve* should be NLT 0.99.

Percent recovery of the Check standard: 80%–120%

Relative standard deviation: Inject 3 replicates of the *Standard solution* prepared at 10 ng/mL, and record the histamine peak areas. The percent relative standard deviation (%RSD) of the histamine peak areas is NMT 10%. If the sample MS signals are lower than the *Standard curve*, the %RSD is not required.

Signal-to-noise ratio: NLT 10 of the histamine peak, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Inject 1 µL of the *Sample solution*, and record chromatograms and peak area of MS signals.

Calculation: From the *Standard curve* obtained and the histamine peak area of the *Sample solution*, determine the histamine concentration, *C*, in the *Sample solution*. Calculate the content of histamine, in ng/mg (ppm), in the gentamicin sulfate sample:

$$\text{Result} = C/S$$

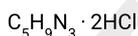
C = concentration of histamine in the *Sample solution* (ng/mL)

S = concentration of gentamicin sulfate in the *Sample solution* (mg/mL)

ADDITIONAL REQUIREMENTS

- [USP REFERENCE STANDARDS \(11\)](#)

[USP Histamine Dihydrochloride RS](#)



184.07▲ (USP 1-May-2022)

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
<426> HISTAMINE TEST METHOD	Ying Han Associate Science & Standards Liaison	BIO42020 Biologics Monographs 4 - Antibiotics

Most Recently Appeared In:

Pharmacopeial Forum: Volume No. 46(4)

Current DocID: [GUID-6F14AF8A-6F25-4FBD-AF0B-70FBC33A16FB_2_en-US](#)

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