Poster Reprint

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A Simple Cost-Effective Determination of Histamines in Gentamicin by HPLC/MS

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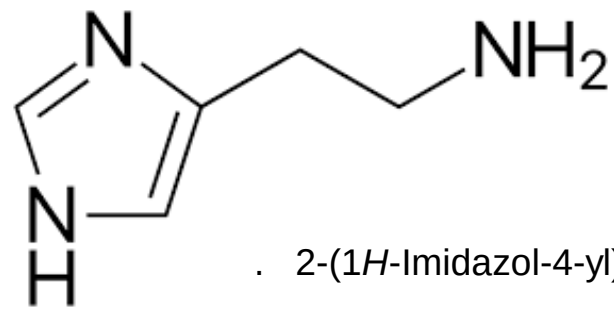
Introduction

Gentamicin is a mixture of related aminoglycosides, the sulfate salt of which is used as a broad-spectrum antibiotic in the treatment of severe infections of organisms such as *Staphylococcus*, *Klebsiella* and others. Intravenous and intramuscular application of this antibiotic treatment is necessary for most systemic applications, although topical applications can be effective for local infections (skin, eyes). *[1]

Part of the gentamicin manufacturing process involves growing gentamicin-producing bacteria in a nutrient-rich growth medium that includes peptone, an ingredient derived from fish. Elevated histamine levels in the Gentamicin were linked to poor-quality fish peptone, caused by improper handling and storage conditions. Since gentamicin isn't the only antibiotic that uses fish peptone in its manufacturing process, other antibiotics might also contain elevated histamine levels that could lead to adverse reactions in patients. Various well documented methods exist for the quantitation of histamines in food matrices. These include enzymatic and colorimetric tests, ELISA, spectrofluorometric and HPLC methods. However, these are often time-consuming and require derivatization. Methods suitable for use in pharmaceutical applications are also well documented but require expensive detectors and highly skilled analysts. The novel approach presented in this method utilizes standard chromatographic methods and simple cost-effective equipment without sacrificing accuracy for the screening and quantitation of histamines in Gentamicin matrices.

Experimental



. 2-(1*H*-Imidazol-4-yl)ethanamine

Equipment

All experiments in this study were performed on the Agilent Pro iQ Plus (P/N G6170A) mass detector with electrospray ionization coupled with an Agilent 1290 Infinity III Multisampler (G7167B), an Agilent 1290 Infinity III Flexible pump (G7120A), and an Agilent 1290 Infinity III Multicolumn Thermostat (G7116B). The system was controlled by Agilent OpenLab CDS software, version 2.8 feature pack e. Data processing was performed using the same Agilent OpenLab CDS software.

Samples, Standards and Consumables:

Histamine, Supelco PN 59964, CAS Number 51-45-6

Gentamicin Sulfate, Sigma-Aldrich PN G1914, CAS number 1405-41-0

Agilent InfinityLab LCMS grade Water, PN 5191-5121, CAS Number 7732-18-5

Agilent InfinityLab LCMS grade Acetonitrile, PN 5191-5101

Agilent Ammonium Formate, PN G1946-85021, CAS 540-69-2

Agilent InfinityLab Poroshell 120 HILIC-Z 3.0x100 2.7 micron Column, PN 685975-324

Experiment

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Parameter	Setting
Analytical Column	Agilent HILIC-Z 3.0x100mm 2.7 µm
Column Oven	30.0°C
Injection Volume	0.5 µl
Run Time	20.00 min.
Post-run Time	10.00 min.
Flow Rate	0.7 ml/min

Channel	Solvent
A1	Water + 0.1% Ammonium Formate
B1	Acetonitrile
A2	Not Used
B2	Not Used

Time	A1	B1
0 Min	20.00%	80.00%
5 Min	20.00%	80.00%
15 Min	35.00%	65.00%

Pro iQ Plus Parameters

Parameter	Value
Gas Temperature (°C)	300
Drying Gas Flow (l/min)	14
Nebulizer (psig)	40
Capillary Voltage	3500

Figure 1. A picture of the Agilent Infinity III Pro iQ Plus LC/MSD

Sample preparation

A 1000 ppm solution of Gentamicin standard was made from 1.206 g gentamicin sulfate Gentamicin powder, 1405-41-0 (sigmaaldrich.com) which was added to 998 g water to prepare the diluent. A 15,000 ppb solution was made from histamine standard (Supelco PN 59964, CAS Number 51-45-6) and diluted by a factor of 10 to make the 1500 ppb standard. The 1500ppb standard was diluted further by a factor of 10 to make the 150 ppb standard. Both standards were utilized for the calibration curve (load on column method) as well as samples to measure accuracy and precision.

Results and Discussion

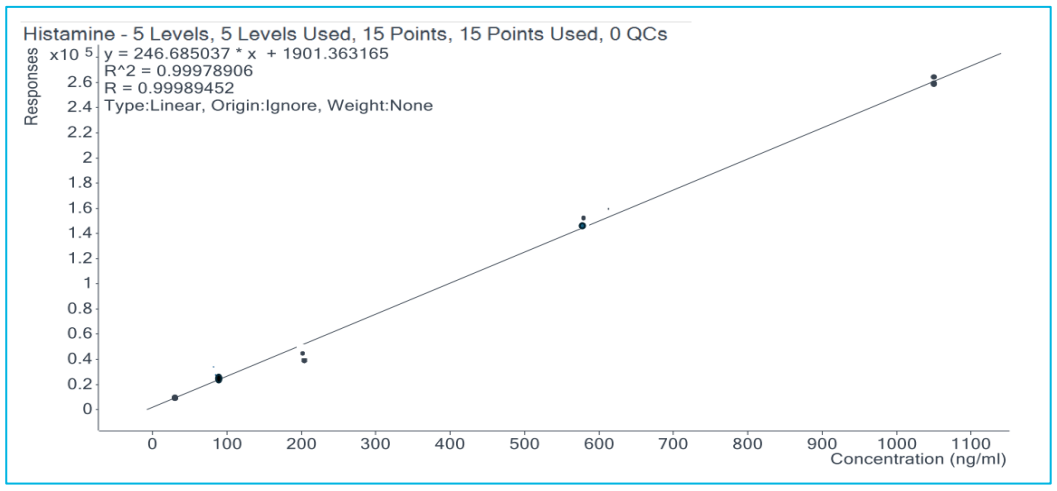

Figure 2. Calibration curve of histamine in a gentamicin matrix.

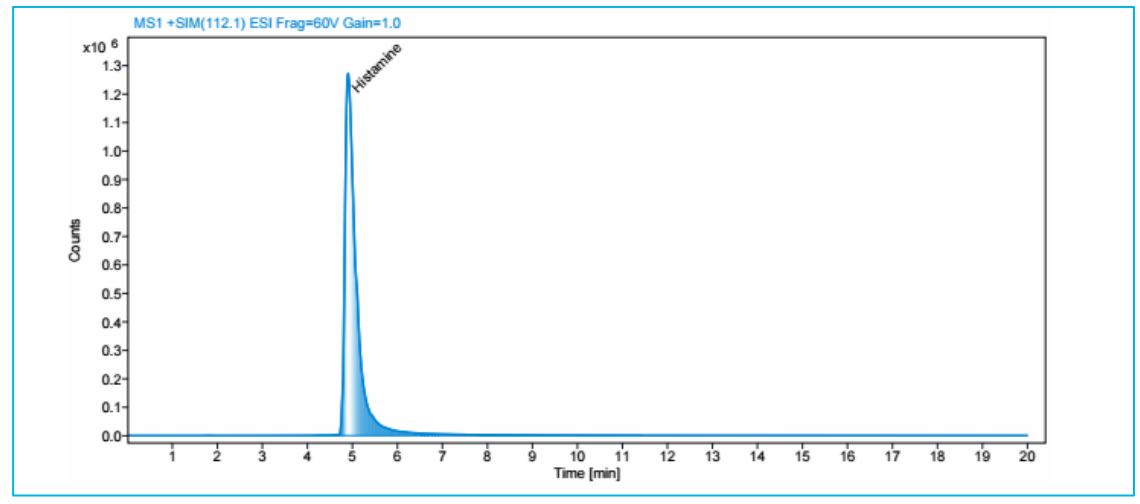

Figure 3. A representative histamine extracted ion chromatogram.

Parameter	Value
Slope	246.68
R	0.9997
R^2	0.9998
Range (ppm)	30 ppb – 1050 ppb

Accuracy

Concentration (ppb)	Cal ppb	Accuracy %
75	76.171	101.562
300	300.574	100.191
450	447.982	99.552
750	748.464	99.795
900	903.411	100.379
Mean		100.296
STD DEV		0.779
%RSD		0.78%

Replicates

Name	RT (min)	Area	Concentration ppm
Histamine (750ppb)	4.066	202954	757.14
	4.082	203130	758.371
	4.074	202223	754.9804
	4.09	202520	756.0897
	4.09	203103	758.2728
	4.098	202617	756.4541
	4.115	205122	765.8213
	4.115	203290	758.9714
	4.123	205059	765.5853
	4.123	204195	762.3532
Mean	4.0976	203421.3	759.4614
STD	0.020576	1026.861	3.839822
%RSD	0.502151	0.504795	0.505598

Conclusions

The Agilent Pro iQ Plus (G6170A) mass detector yields excellent linearity, accuracy and precision in the detection and quantitation of histamines in Gentamicin. The extensive linear range allows for detection of histamines in Gentamicin at extremely low levels.

This method is a simple, accurate and cost-effective way to screen and quantitate histamine contamination in Gentamicin.

References

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2. Maria Cristina Gaudiano, Luisa Valvo, Andrea Luca Rodomonte, A Q-TOF LC/MS method for identification and quantitation of Histamine in the antibiotic Gentamicin at ppm level: Validation and uncertainty evaluation, Journal of Pharmaceutical and Biomedical Analysis, Volume 162, 2019, Pages 158-163, ISSN 0731-7085, https://doi.org/10.1016/j.jpba.2018.09.015

3. Antibiotics manufacturing components can trigger adverse reactions. Standards help restore quality and trust, https://qualitymatters.usp.org/antibiotics-manufacturing-components-can-trigger-adverse-reactions-standards-help-restore-quality. *Medicines* Dec.10, 2019

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