Assessment of Commercially Available Devices for the Removal of Histamine from Red and White Wines

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INTRODUCTION

Wine has been produced for thousands of years and it is one of the most commonly consumed alcoholic beverages around the globe. Histamine is produced in wine during fermentation, as naturally occurring histidine from the grapes is decarboxylated to histamine.

In spite of its popularity, there is a portion of the population who do not tolerate histamine well and may encounter unpleasant side effects such as itchy watery eyes, rhinorrhea, headaches and flushing. There are many commercially available products claiming to remove the histamine while preserving the quality of the beverage taste and aroma. Directions are generally to swirl the device in a glass of wine or pour the wine through a pour spout to remove the histamine.

The purpose of these research was to validate a method that could measure histamine in red and white wine and evaluate the efficacy of histamine removing devices.

METHODS

Quantitative Analysis:

•Agilent 1200 HPLC coupled to an Agilent 6430 triple quadrupole (LC/MSMS)
•Analytical column: Phenomenex **HILIC** column @ 40°C
- Cogent Diamond Hydride 100A (4 µm, 150 x 2.1 mm)

•MPA: 0.1% Formic Acid in Water
•MPB: 0.1% Formic Acid in Acetonitrile
•Injection volume 2 µL
•MSMS using Positive ESI

Time (min)	Flow (mL/min)	% A	% B
Initial	0.4	60	40
3	0.4	90	10
5.5	0.4	90	10
6	0.4	60	40
9.5	0.4	60	40

•Data processing Agilent Masshunter® Quantitative v10.1

Histamine MRM:

Compound	Precursor	Product Ion	Dwell	Fragmentor	CE
Histamine-d4	116	99.1	100	82	12
		85.1	100	82	16
Histamine	112	95.1	100	90	16
		68.1	100	90	24

Method Validation

Fit-for-purpose 3-day validation adapted from the American Standards Board (ASB) validation standard including:

•Limit of detection (LOD), Lower limit of quantitation (LLOQ), Calibration model, Bias & precision, interferences with common drugs, ion suppression and enhancement, dilution integrity.
•Calibration range 5-2000 ng/mL in 2 matrices
- Red Grape Juice **RGJ** and in White Grape Juice **WGJ**.

Sample preparation

Samples were not extracted but prepared by simple dilution and filtration.
- Red wine samples 1:10 dilution (due to the high concentration of histamine)
- White wine samples 1:5 dilution.
- Matrix match Red Grape Juice (**RGJ**) and White Grape Juice (**WGJ**)
- All samples are filtered using a 0.2µm nylon filter using a 1 mL Luer-lock syringe to apply pressure, 1 drop/sec.

1. 1000 µL of MPB 60:40.
2. 100 µl **RGJ** or 200 µL of **WGJ** for calibrators and QCs
3. 100 µL Red Wine or 200 µL White Wine of sample to be analyzed.
4. 50 µL of Histamine-d4 @ 1ng/µL

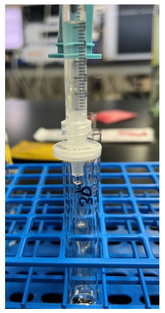


Figure 1 – White Wine Sample being filtered.

VALIDATION RESULTS & EXPERIMENT SETUP

Validation results:

LOD and LLOQ = 5 ng/mL
Calibration model: 5 - 2000 ng/mL
- **RGJ** R^2 0.99935
- **WGJ** R^2 0.99936

Bias and precision: <15%

No interferences at 1000ng/mL for the following analytes:
- Putrescine
- Cadaverine
- L-Histidine
- Tyramine
- Spermidine
- Tryptamine

Experiment Set up:

Three red wines and three white wines were evaluated at 4 different times:
- 0 min/ T_0 Control (prior use of device)
- 3 min
- 5 min
- 30 min

Wine volume used: 200 mL per device
Aliquoted 3 mL of sample at every time stamp. Each sample run in triplicate.
Total number of samples analyzed:
- 108 red wine aliquots
- 108 white wine aliquots.

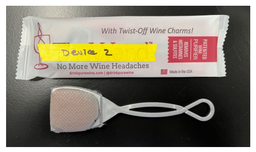
Figure 2. - Red Wines evaluated

All 3 devices were evaluated to determine:
- What device removes the most histamine
- If the histamine removed matches the time recommended by the manufacturer
- Evaluation of a longer time for the removal of Histamine

Figure 3 - White Wines evaluated

HISTAMINE REMOVING DEVICES

Device 1 –
- Shaped as a tea bag.
- Device user manual: hold from the string and submerged in the wine for **5 min**.
- Volume recommended: **6 to 8 fl oz (177 mL to 236 mL)**
- Percentage of histamines and sulfites stated to remove: **Not provided**



Device 2 –
- Shaped as a wand with a tea bag in one end.
- Device user manual: Stir in wine for **3 min**.
- Volume recommended: **Not Stated**
- Percentage of histamines and sulfites stated to remove: **95%**

Device 3 –
- Shaped as a tea bag without a string attached.
- Device user manual: Stir in wine for **5 min**.
- Volume recommended: **6 fl oz (177 mL)**
- Percentage of histamines and sulfites stated to remove: **Not provided**

RESULTS RED WINE vs WHITE WINE

All red wines (Wine A, B and C) had concentrations between 5 and 2000 ng/mL in a 1:10 dilution.
From the white wines, only one (Wine E) had enough histamine to be detected, after a 1:5 dilution. All 3 devices were evaluated to determine the amount of histamine they were able to remove.

Wine A (Red)	Device 1		Device 2		Device 3	
	Average (ng/mL)	Percent Differ	Average (ng/mL)	Percent Differ	Average (ng/mL)	Percent Differ
T_0	5614.11	-	5614.11	-	5614.11	-
3 min	5603.00	-0.20%	5542.33	-1.29%	5658.67	0.79%
5 min	5541.33	-1.30%	5506.00	-1.94%	5496.33	-2.12%
30 min	5545.33	-1.23%	5346.33	-4.89%	4993.67	-11.70%

Wine A was measured a concentration of 5600 ng/mL
- **Device 1** removed up to 1.3%
- **Device 2** removed up to 4.8%
- **Device 3** removed up to 11.7%

Device 3 removed the most histamine, needing 30 min to remove at least 10% of initial histamine.

Wine B was measured a concentration of 3700 ng/mL
- **Device 1** removed up to 4.1%
- **Device 2** removed up to 6.0%
- **Device 3** removed up to 12.4%

Again, device 3 is the one that has removed the most amount of histamine, needing 30 min to remove at least 12% of initial histamine

Wine B (Red)	Device 1		Device 2		Device 3	
	Average (ng/mL)	Percent Differ	Average (ng/mL)	Percent Differ	Average (ng/mL)	Percent Differ
T_0	3706.56	-	3706.56	-	3706.56	-
3 min	3687.00	-0.53%	3473.67	-6.49%	3599.33	-2.94%
5 min	3688.50	-0.49%	3555.00	-4.17%	3589.00	-3.22%
30 min	3557.00	-4.12%	3489.33	-6.04%	3273.33	-12.41%

Wine C (Red)	Device 1		Device 2		Device 3	
	Average (ng/mL)	Percent Differ	Average (ng/mL)	Percent Differ	Average (ng/mL)	Percent Differ
T_0	2394.33	-	2394.33	-	2394.33	-
3 min	2341.67	-2.22%	2381.67	-0.53%	2363.67	-1.29%
5 min	2440.67	1.92%	2316.50	-3.30%	2372.67	-0.91%
30 min	2382.33	-0.50%	2314.67	-3.38%	2216.00	-7.74%

Wine C was measured a concentration of 2400 ng/mL
- **Device 1** removed up to 0.5%
- **Device 2** removed up to 3.3%
- **Device 3** removed up to 7.7%

Again, device 3 removed the most histamine. Device 1, from initial measurements removes at least 2%, but after 30min, data suggest almost no histamine removal.

Wine E was measured a concentration of 62 ng/mL
- **Device 1** removed up to 5.2%
- **Device 2** removed up to 6.9%
- **Device 3** removed up to 15%

Again, device 3 was the one that removed the highest percentage of histamine.

Wine E (White)	Device 1		Device 2		Device 3	
	Average (ng/mL)	Percent Differ	Average (ng/mL)	Percent Differ	Average (ng/mL)	Percent Differ
T_0	62.72	-	62.72	-	62.72	-
3 min	61.33	-2.24%	60.17	-4.16%	58.83	-6.40%
5 min	61.83	-1.43%	61.00	-2.78%	58.17	-7.54%
30 min	59.50	-5.27%	58.50	-6.97%	53.83	-15.25%

DISCUSSION AND CONCLUSION

Histamine adverse effects affect a large percent of the population who suffer from histamine intolerance. Commercial products are sold with the promise of removing histamine and sulfites that will help sensitive individuals.

This project has successfully shown the ability to measure histamine in different kinds of red and white wines, and the evaluation of 3 readily available devices that promise the removal of histamine. Device 3 has shown best result at removing histamine in red and white wine after 30 min (25 min longer than recommended).

The results suggest that these devices do not remove as much histamine as it is promised, even though public opinions about the different products suggest lesser effects after its usage. This research does not evaluate the removal of sulfites, so it is not able to assess if the diminished effects suffered by the public are due to the sulfite removal with these devices or a placebo effect.

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