Trace level detection of histamine as low as 0.1 mg/l in wine using RIDASCREEN® Histamine (enzymatic)

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Introduction

It was reported that a few susceptible consumers already reacted to 15 - 30 µg histamine per serving size of wine (200 ml). This is equivalent to a concentration of 0.08 - 0.15 mg/l histamine. Frequently HPLC is used to detect histamine in wine. However, at low concentration the method is not reliable due to disturbing peaks. Also the HPLC method is very cumbersome.
Our RIDASCREEN® Histamine (enzymatic) has a claimed LOD of 0.54 mg/l which is not sensitive enough to detect these low concentrations of histamine. Therefore, we developed a sensitive application to cover these needs.

Picture 1: RIDASCREEN® Histamine (enzymatic), Art. No. R1605

Results

Before using the RIDASCREEN® Histamine (enzymatic), R1605, for quantification of histamine in wine, the samples need to be extracted by using RIDA® Sample Decolorant (R1699) which removes interferences from red and white wine. For the more sensitive measurement procedure, the extraction procedure using RIDA® Sample Decolorant remains the same except all pipetted volumes are doubled. The enzymatic procedure was changed by increasing the calibrator/sample volume from 100 µl to 200 µl and reducing the buffer volume from 150 µl to 50 µl. Due to these changes the calibrator concentrations need to be changed by the user using the calibrators from the test kit. The new linear range of the test kit is from 0.2 to 5 mg/l histamine (originally 1 mg/l up to 20 mg/l). For easy calculation of results, a special software, RIDASOFT® Win.NET is available. This software does also contain a residual plot analysis to check for suitable linearity of the calibration graph.

Methods

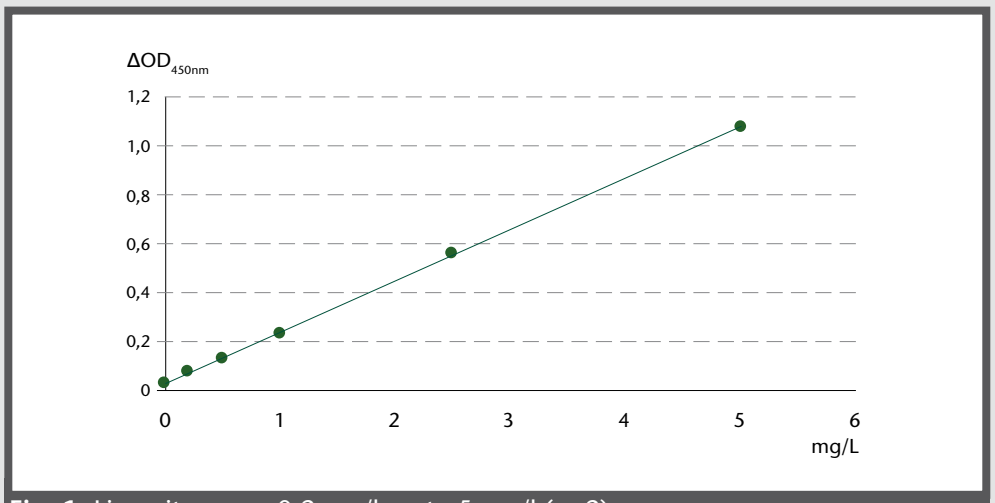

Fig. 1: Linearity range 0.2 mg/l up to 5 mg/l (n=2)

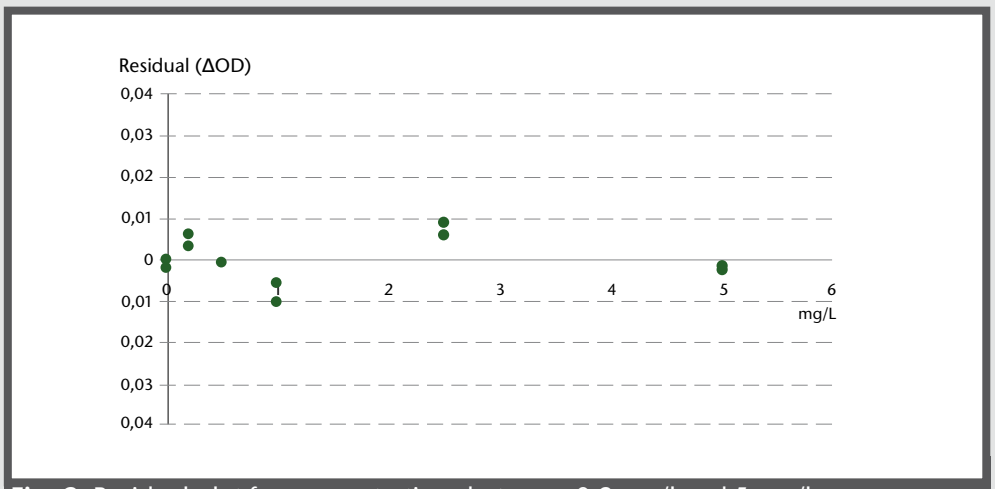

Fig. 2: Residual plot for concentrations between 0.2 mg/l and 5 mg/l

Using this new procedure, the LoD and LoQ were decreased to 0.19 and 0.34 mg/l histamine in wine, respectively (following DIN ISO 11843-2:2008-06). These values were calculated from low concentrated calibration solutions. To confirm especially the LoQ, a wine sample with a mean histamine concentration of 0.14 mg/l was analyzed and showed a CV of 38 % (table 1) while spiking this wine with 0.5 mg/l resulted in a mean concentration of 0.35 mg/l (CV of 6.9 %) after subtraction of the blank level.

Table 1: Confirmation of the limit of quantification

Rep.	Spiked histamine concentration (mg/l)				
	0	0.25	0.5	0.75	1
1	0.11	0.20	0.32	0.53	0.70
2	0.09	0.18	0.35	0.51	0.72
3	0.23	0.16	0.39	0.53	0.74
4	0.14	0.13	0.34	0.55	0.70
5	0.09	0.15	0.34	0.60	0.74
6	0.16	0.18	0.34	0.53	0.74
Mean	0.14	0.17	0.35	0.54	0.72
SD	0.05	0.03	0.02	0.03	0.02
CV%	38.2	15.9	6.9	5.6	2.4

Recoveries at concentrations between 0.5 mg/l and 10 mg/l varied between 73 % and 118 % (table 2). Especially at low concentrations the recovery calculation was beset with some uncertainties since the red wine used for spiking showed a mean concentration of 0.99 mg/l but a quite high CV.

Table 2: Recovery of histamine in red wine

Rep.	Spiked histamine concentration (mg/l)				
	0	0.5	2	5	10
1	1.02	1.50	2.49	6.07	10.00
2	1.01	1.54	2.36	6.01	9.79
3	0.87	1.60	2.31	5.92	10.04
4	0.99	1.46	2.59	6.03	10.03
5	0.94	1.66	2.57	5.96	10.03
6	1.09	1.69	2.36	5.98	9.82
Mean	0.99	1.57	2.45	6.00	9.95
SD	0.08	0.09	0.12	0.05	0.12
CV%	7.8	5.7	4.8	0.9	1.2
Mean rec.		118 %	73 %	100 %	90 %

Nevertheless, the results clearly show that the new procedure is capable in measuring the challenging concentrations. The procedure is robust and not time consuming as an HPLC method.

07/2017