

# HPLC Application

ID No.: 20869

## OPA Derivatization in Wine of Histadine and Histamine using Kinetex 2.6u C18

**Column:** Kinetex<sup>®</sup> 2.6  $\mu$ m C18 100 Å, LC Column 100 x 4.6 mm, Ea  
**Dimensions:** 100 x 4.6 mm ID  
**Order No:** 00D-4462-E0  
**Elution Type:** Gradient  
**Eluent A:** 20mM Sodium Acetate pH 7.2 w/0.03% Sodium Azide (pre-filtered 0.2um)  
**Eluent B:** 50:50 Acetonitrile/Methanol

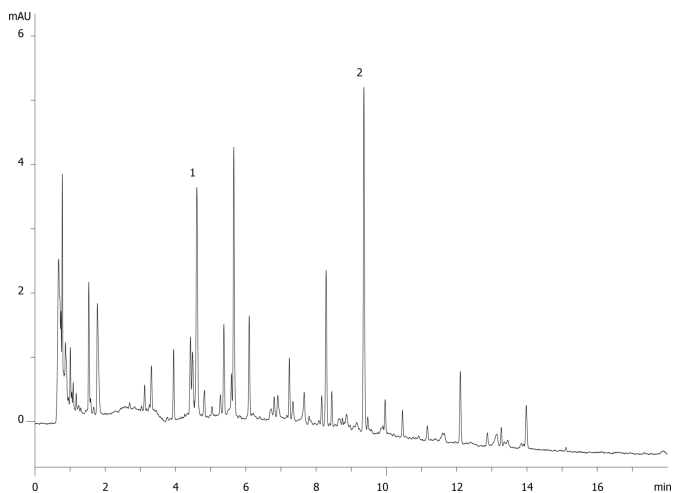
Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	97	3
	2	20	20	80
	3	20.5	97	3
	4	23	97	3

**Flow Rate:** 1.2 mL/min  
**Col. Temp.:** 30 °C  
**Detection:** UV-Vis Abs.-Variable Wave.(UV) @ 338 nm (ambient)

**Detector Info:** Offline Amino Acid derivatization:

1. In to a amber vial dispense 250uL of 20mM Sodium Acetate pH 12.5
2. Add 100uL of Sample (ex: Wine sample, Standards)
3. Mix for 30 seconds
4. Add 50uL of OPA derivatization agent (OPA: Part # 5061-3335)
5. Mix vigorously for 30 seconds
6. Add 2.0mL of MilliQ Water
7. Mix for 30 seconds
8. Inject 10uL of sample on to system

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Products used in this application:



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### ANALYTES:

- 1 Histidine
- 2 Histamine

