Application Note

Food Testing &
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1290 Infinity III LC

# Analysis of Biogenic Amines in Wine Using the Agilent 1290 Infinity II LC and Ultivo Triple Quadrupole LC/MS

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## Abstract

This application note demonstrates the powerful combination of an Agilent 1290 Infinity II LC with an Agilent Ultivo triple quadrupole LC/MS for direct analysis of biogenic amines in wine. As the stackable design of the LC/MS system eliminates the usual MS footprint, measurements could be conducted using a small lab space. Agilent MassHunter workstation software was used for automated optimization of ESI source and MRM parameters resulting in sensitive and selective detection of target compounds. The presented method enables high-throughput reproducibility as well as a chromatographic approach with moderate backpressure, enabling the application of lower-end LC systems such as the 1260 Infinity II Prime LC. Biogenic amine analysis in Tempranillo red wine as well as performance parameters (e.g., limit of detection, calibration linearity, and precision) are shown.

## Introduction

Biogenic amines are low molecular weight nitrogenous organic bases that are naturally occurring in living beings and take part in several cellular functions. As biogenic amines can be formed by bacterial decarboxylation of amino acids or fermentation processes, they are abundant in protein-rich and fermented products. Elevated concentrations of biogenic amines can be an indicator for poor hygienic conditions, and intake of high amounts of biogenic amines can even have toxic effects on organisms.[1]

Analytical methods for the determination of biogenic amines reported in literature mainly rely on derivatization with chromogenic or fluorogenic dyes, because they show insufficient retention on reversed-phase columns as well as weak fluorescence or ultraviolet absorbance. However, most derivatizations are not specific, resulting in unwanted coelution of derivatized matrix components and target analytes. Due to the structural variety of biogenic amines and matrix components, analytes can show diverse reactivities and result in different or incomplete derivatization products. Additionally, derivatization can be a costly and time-consuming task. Consequently, direct analysis of underivatized biogenic amines enabling high throughput, as well as sensitive and selective detection, would be more desirable.[2,3,4]

As some countries have already set limits (e.g., for histamine) in food, there is a need for analytical methods ensuring compliance with concentration limits of biogenic amines. This application note demonstrates the use of a 1290 Infinity II LC for direct analysis of biogenic amines in wine using ion pair chromatography prior to MS analysis on an Ultivo LC/TQ.

## Experimental

### Instrument:

- Agilent 1290 Infinity II flexible pump (G7104A) without mixer
- Agilent 1290 Infinity II vialsampler (G7129B) with 40 µL analytical head and 20 µL loop
- Agilent 1290 Infinity II multicolumn thermostat (G7116B) with standard heat exchanger (G7116-60015)
- 0.12 mm id system capillaries
- Agilent Ultivo LC/TQ (G6465B)

### Software

- Agilent MassHunter workstation
  - LC/MS data acquisition for Ultivo LC/TQ, V1.1
  - Optimizer for LC/TQ, V1.1
  - Source Optimizer for LC/TQ, V1.1
- Agilent MassHunter Qualitative Analysis software, V10.0
- Agilent MassHunter Quantitative Analysis software, V10.0

### Column

Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm (part number 699775-902)

| HPLC Method | |
|---|---|
| Solvent | A) Water + 0.1% heptafluorobutyric acid<br>B) Acetonitrile + 0.1% heptafluorobutyric acid |
| Timetable | 0 min: 2% B;<br>0.5 to 7 min: 2 to 30% B;<br>7.5 to 7.8 min: 80% B;<br>8 min: 2% B |
| Stop Time/Post-Time | 8.1 min/2 min |
| Flow Rate | 0.8 mL/min |
| Injection Volume | 1 µL |
| Column Temperature | 30 °C |
| Sample Temperature | Ambient |
| Needle Wash | 5 s in flush port, wash solvent: 0.1 M HCl in water/acetonitrile (1:1) |
| Pressure Limits | Minimum: 0 bar; maximum: 600 bar |
| **MS Method** | |
| Ion Source | Agilent Jet Stream |
| Drying Gas Temperature/Flow | 200 °C/13 L/min |
| Sheath Gas Temperature/Flow | 350 °C/12 L/min |
| Nebulizer | 45 psi |
| Capillary/Nozzle Voltage | 4,000 V/0 V |
| Scan Mode | Dynamic MRM (detailed parameters: see Table 1) |
| Polarity | Positive |
| Time Filter Window | 0.05 min |
| MS1 Resolution, MS2 Resolution | Unit |
| Timetable | Diverter to waste after 7 min |

## MS method optimization

Agilent MassHunter Optimizer for Ultivo LC/TQ (Version 1.1) was used for automated optimization of collision energy (5 to 50 V) and fragmentor voltage (40 to 200 V) of precursor and product ions. Therefore, analytical standards were separately injected using the 1290 Infinity II vialsampler without application of a chromatographic column.

MassHunter Source Optimizer for Ultivo LC/TQ (Version 1.1) was used to find parameters for beneficial ESI settings. The most abundant MRM transition was used to optimize the following parameters: sheath gas temperature from 100 to 400 °C (50 °C steps); sheath gas flow from 8 to 12 L/min (1 L/min steps), gas temperature from 200 to 350 °C (30 °C steps); gas flow from 5 to 13 L/min (2 L/min steps); nebulizer from 20 to 50 psi (5 psi steps); capillary from 2,000 to 5,000 V (500 V steps); nozzle voltage from 0 to 2,000 V (500 V steps).

## Standards

- Agmatine sulfate, cadaverine dihydrochloride, dopamine hydrochloride, 1-heptylamine, 1-hexylamine, histamine, isopentylamine, 2-methylbutylamine, L-norepinephrine hydrochloride, putrescine, serotonin hydrochloride, spermidine, spermine, tryptamine, and tyramine were obtained from Thermo Fisher GmbH, Germany and VWR International GmbH, Germany.
- All solvents were purchased from Merck, Germany.
- Fresh ultrapure water was obtained from a Milli-Q integral system equipped with LC-Pak polisher and a 0.22 µm membrane point of use cartridge (Millipak).

**Table 1.** Dynamic MRM settings (bold blue: quantifier product ion).

| Compound | RT (min) | RT Window (min) | Precursor Ion (*m/z*) | Product Ions (*m/z*) | Collision Energy (eV) | Fragmentor (V) |
|---|---|---|---|---|---|---|
| Agmatine | 2.7 | 1 | 131.1 | **71.8**, 59.9, 113.8 | 17, 9, 9 | 92 |
| Cadaverine | 2 | 1 | 103.1 | **85.8**, 41, 68.8 | 5, 21, 13 | 72 |
| Dopamine | 1.8 | 1 | 154.1 | 90.8, **136.6**, 64.8 | 25, 5, 41 | 82 |
| Heptylamine | 6.1 | 1 | 116.2 | **56.9**, 40.9, 42.9 | 9, 21, 17 | 87 |
| Hexylamine | 4.9 | 1 | 102.1 | **43**, 40.9, 84.8 | 13, 25, 5 | 72 |
| Histamine | 2.2 | 1 | 112.1 | **94.8**, 67.8, 40.9 | 13, 21, 29 | 92 |
| Isopentylamine | 3.4 | 1 | 88.1 | **43**, 70.9, 40.9 | 13, 5, 21 | 72 |
| 2-Methylbutylamine | 3.2 | 1 | 88.1 | **42.9**, 70.9, 41 | 13, 5, 21 | 67 |
| Norepinephrine | 0.6 | 1 | 170.1 | **151.9**, 106.8, 76.9 | 5, 21, 41 | 62 |
| Putrescine | 1.7 | 1 | 89.1 | **71.9**, 30, 54.8 | 5, 21, 21 | 52 |
| Serotonin | 3 | 1 | 177.1 | **159.6**, 114.7, 116.8 | 5, 29, 33 | 52 |
| Spermidine | 4.5 | 1 | 146.2 | **71.8**, 128.9, 83.9 | 17, 5, 25 | 92 |
| Spermine | 5.9 | 1 | 203.2 | **111.8**, 83.8, 128.7 | 21, 37, 9 | 89 |
| Tryptamine | 4.8 | 1 | 161.1 | **143.7**, 116.7, 142.8 | 5, 25, 29 | 72 |
| Tyramine | 2.5 | 1 | 138.1 | **120.7**, 76.8, 90.8 | 5, 29, 25 | 57 |

## Sample preparation

- Standards substances were dissolved and diluted in 0.1 M HCl in water.
- Tempranillo red wine was obtained from a local store and filtered before LC/MS analysis using a 15 mm Agilent Captiva premium syringe filter with 0.2 µm regenerated cellulose membrane (part number 5190-5108).
- Samples were transferred into vials with polypropylene inserts (part number 5182-0549).

## Calibration/limit of detection/matrix effects

Calibration was conducted using 0.2 nM to 100 µM of standard substances diluted in 0.1 M HCl in water as well as in wine for calculation of matrix effects. Individual calibration ranges were set for each target compound in accordance with signal responses of the wine sample. Limit of detection (LOD) was determined using the formula from ICH validation guideline Q2(R1), where σ is the standard deviation of the response and S is the slope of the calibration curve:

$$\text{LOD} = 3.3 \times \frac{\sigma}{S}$$

Matrix effects (ME) were determined using the following formula:

$$\%\text{ME} = \frac{\text{Slope (matrix-matched)}}{\text{Slope (solvent)}} \times 100$$

## Results and discussion

As underivatized amines usually show little retention on reversed-phase columns, ion pair chromatography was used to gain separation of target substances. Application of heptafluorobutyric acid as an ion pair reagent and an InfinityLab Poroshell 120 EC-C18 (2.1 × 50 mm, 2.7 µm) column showed acceptable chromatographic separation and run time (Figure 1A). Even separation of the structural isomers 2-methylbutylamine and isopentylamine could be achieved by this chromatographic approach. Target compounds eluted in less than 7 minutes and between 2 and 30% B, allowing a very low consumption of organic solvent for the relevant retention window. Depending on what sample type and preparation is used, the organic solvents can be ramped up after 7 minutes to allow elution of irrelevant matrix components. In this case, the organic solvent was ramped up to 80% B, as described in the HPLC method section. Depending on the complexity of sample matrix, application of an HPLC guard to reduce particulates and chemical contamination for extending analytical column lifetime could easily be handled in terms of backpressure, which was below 400 bar during sample runs. Method reproducibility was examined by calculation of relative standard deviations of peak areas and retention times (RT) using 10 consecutive injections of an analytical standard mixture. Area precision ranged from 0.5 to 1%; RT precision was less than or equal to 0.002% for several compounds, while a few showed values up to 0.237% (Table 2).

Individual calibration ranges were chosen in accordance with the analyte response in wine (Table 2), and calibration showed linear responses with $R^2$ values of 0.99 for most of the compounds. LODs were determined based on calibration with analytical standards in 0.1 M HCl in water and showed values as low as 2 ng/mL (Table 2). Calibration based on analytical standards spiked into a Tempranillo red wine was used for calculation of matrix effects. Most biogenic amines showed only little signal suppression or enhancement in red wine (Table 2). However, agmatine and norepinephrine showed a signal suppression of

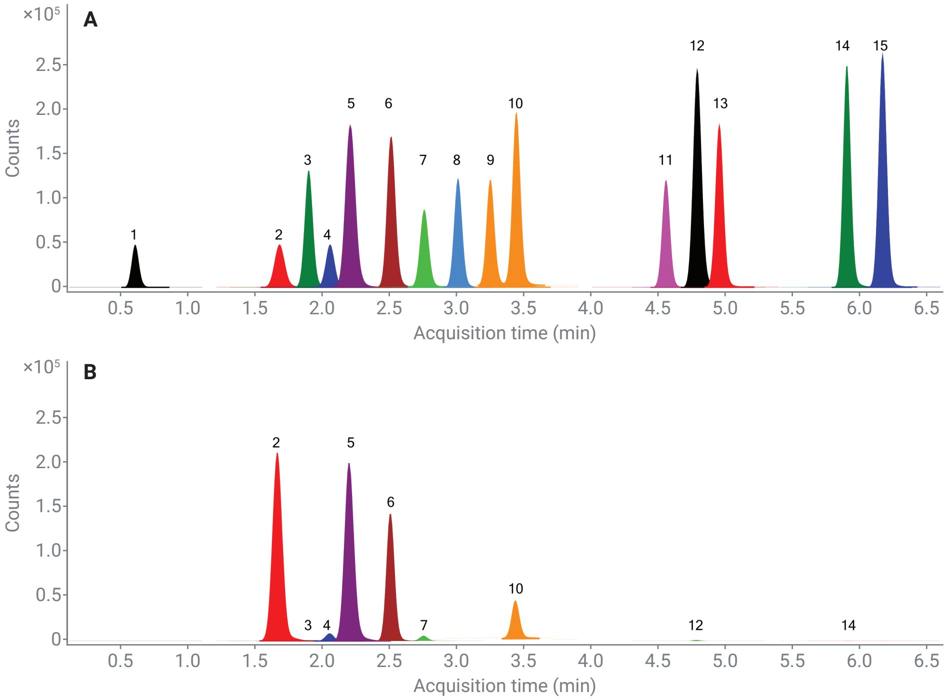

**Figure 1.** (A) Analysis of biogenic amines standard mixture (10 µM of each standard substance in 0.1 M HCl in water) showing overlay of dynamic MRM transitions. (B) Analysis of Tempranillo red wine (1:1 dilution in 0.1 M HCl in water) showing overlay of dynamic MRM transitions. Elution order: norepinephrine (1), putrescine (2), dopamine (3), cadaverine (4), histamine (5), tyramine (6), agmatine (7), serotonin (8), 2-methylbutylamine (9), isopentylamine (10), spermidine (11), tryptamine (12), hexylamine (13), spermine (14), heptylamine (15).

**Table 2.** Summary of results (n.d. = not detectable).

| Compound | Calibration Range | R² | RT Precision (%) | Area Precision (%) | LOD (ng/mL) | ME (%) | Concentration in Wine (mg/L) |
|---|---|---|---|---|---|---|---|
| Agmatine | 6.5 to 650 µg/L | 0.998 | 0.130 | 0.8 | 36 | 55.5 | 0.115 |
| Cadaverine | 0.01 to 5.11 mg/L | 0.995 | 0.220 | 0.7 | 419 | 104.5 | 0.256 |
| Dopamine | 0.77 to 76.6 µg/L | 0.975 | 0.237 | 0.8 | 16 | 83.2 | 0.008 |
| Heptylamine | 0.02 to 5,761 µg/L | 0.991 | 0.001 | 0.5 | 470 | 94.9 | n.d. |
| Hexylamine | 0.51 to 5,059 µg/L | 0.990 | 0.001 | 0.5 | 504 | 93.8 | n.d. |
| Histamine | 0.22 to 11.12 mg/L | 0.987 | 0.200 | 0.7 | 1,767 | 87.5 | 2.466 |
| Isopentylamine | 8.72 to 1,743 µg/L | 0.999 | 0.002 | 0.7 | 76 | 87.4 | 0.110 |
| 2-Methylbutylamine | 0.87 to 1,743 µg/L | 0.998 | 0.002 | 0.9 | 78 | 94.9 | n.d. |
| Norepinephrine | 0.02 to 16.92 mg/L | 0.995 | 0.002 | 0.8 | 1,360 | 50.1 | n.d. |
| Putrescine | 4.41 to 8,815 µg/L | 0.984 | 0.163 | 0.8 | 1,210 | 104.7 | 11.845 |
| Serotonin | 0.88 to 17,621 µg/L | 0.983 | 0.002 | 1.0 | 2,259 | 92.1 | n.d. |
| Spermidine | 2.91 to 7,262 µg/L | 0.995 | 0.058 | 0.8 | 559 | 106.1 | n.d. |
| Spermine | 0.2 to 20.23 µg/L | 0.993 | 0.045 | 0.7 | 2 | 108.4 | 0.001 |
| Tryptamine | 1.6 to 80.11 µg/L | 0.991 | 0.092 | 0.5 | 10 | 95.5 | 0.006 |
| Tyramine | 0.14 to 13.72 mg/L | 0.985 | 0.001 | 0.6 | 2,250 | 91.0 | 2.058 |

approximately 50%, which resulted from coeluting matrix components. Signal suppression might be reduced by further sample preparation or dilution, otherwise isotopically labeled structural analogues coeluting with the analytes of interest should be used to account for matrix effects. Full scan analysis of the wine sample showed that many early eluting matrix components can effectively be separated from target substances (Figure 2). As shown in Figure 2, unnecessary contamination of ion source and optics was avoided by switching the diverter valve to waste after 7 minutes run time. If norepinephrine (RT = 0.6 minutes) is not of interest, it is highly recommended to switch the diverter valve to waste until the first compound of interest elutes.

In Tempranillo red wine, a total of nine different biogenic amines could be detected, with putrescine, histamine, and tyramine predominating to a large extent (Figure 1B, Table 2). External calibration resulted in concentrations of up to 12 mg/L (putrescine) of a single biogenic amine in the analyzed red wine sample. According to the European Food Safety Authority (EFSA), histamine and tyramine are the most toxic biogenic amines, and toxicological thresholds of histamine and tyramine are 50 and 600 mg per person per meal, respectively.[1] As calculated concentrations of histamine and tyramine detected in the wine sample were at 2.5 and 2.1 mg/L, high-volume consumption would be needed to reach the toxicological threshold stated by the EFSA. Although histamine and tyramine concentrations strongly vary in Tempranillo wine from different wine-producing regions, the detected concentration levels are in line with concentrations published in literature.[5]

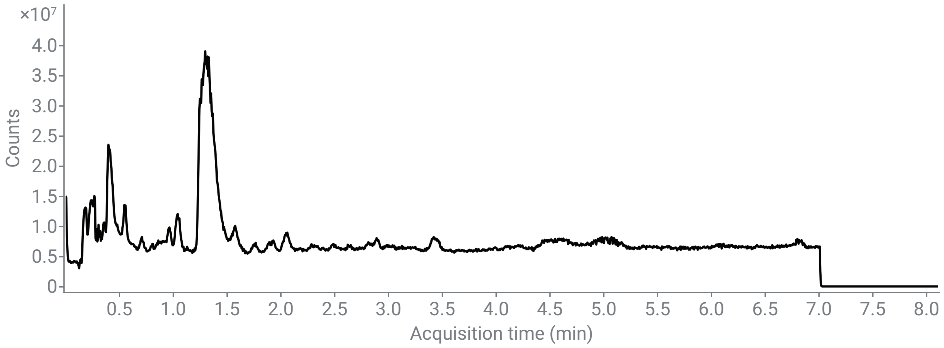

**Figure 2.** Full scan analysis of Tempranillo red wine (1:1 dilution in 0.1 M HCl in water) showing TIC from *m/z* 20 to 1,000 in positive mode.

## Conclusion

This application note demonstrates the use of a 1290 Infinity II LC with an Ultivo LC/TQ mass spectrometer for direct analysis of biogenic amines in wine. The presented method enables high throughput, good area and retention time precision, as well as sensitive and selective detection. Analysis of Tempranillo red wine showed low matrix effects for most target compounds, but should be evaluated if other sample matrices and preparation techniques are used. Considering the scientific opinion of the EFSA, the analyzed Tempranillo red wine is far off the toxicological thresholds of the most toxic biogenic amines.

## References

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