

# Quantification of the biogenic phenethylamine alkaloid hordenine by LC-MS/MS in beer

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## Abstract

**Aim:** The purpose of this study is to use an already established LC-MS/MS method in order to quantify the alkaloid hordenine in various beer brands.

**Methods:** Hordenine was analyzed from 20 µl beer after addition of d<sub>4</sub>-hordenine as internal standard with an Agilent Eclipse XDB-C18, 5 µm, 4.6 x 150 mm column coupled to a Waters Acquity ultra performance LC. Three transitions in multiple reaction monitoring mode (MRM) were used for identification. One transition was applied for quantification. The internal standard d<sub>4</sub>-hordenine was synthesized by methylation of tyramine with deuterium labeled formaldehyde and sodium cyanoborhydride by reductive amination.

**Results and Discussion:** Different types of beer have been examined by LC-MS/MS. Top fermented dark beer (Alt), Pils, Kölsch, wheat beer (Weizenbier) and malt liquor as well as other types of beer from the EU such as mixed beers (Salitos, Desperados) and non-alcoholic beers were analyzed. Up to 2 - 4 mg/L hordenine were found in typical German and regional beers (Alt, Pils, Kölsch); no significant differences between top-fermenting and bottom-fermenting beer types were found. In mixed beers the concentrations were similar to those of wheat beer (around 2 mg/L). The highest concentrations of hordenine (>4 mg/L) were measured in strong beers.

**Conclusion:** Hordenine can serve as a reliable parameter to proof beer consumption.

## 1. Introduction

Hordenine is a common substance extracted from the cactus *Anhalonium fissuratum* first described by Arthur Heffter 1894 [1]. Hordenine was also detected in *Graminaceae*, *Cactaceae* some *Algae* and *Citrus aurantium* [2]. 1906 [3] the detection and structural elucidation of hordenine from an extract obtained from barley (*Hordeum vulgare*) was successful. Hordenine is only detectable in barley during germination. It reaches its maximum concentration after 3-9 days and decreases with increasing age of the seedling [4, 5]. Hordenine is assigned to the family of phenylethylamines. It is synthesized by the enzyme methyltransferase which methylates the precursor tyramine twice.

Hordenine has been demonstrated in various studies that hordenine has a positive inotropic effect on the heart in healthy mice and dogs [6]. Furthermore it inhibits the feeding behaviour of insects and has a diuretic effect on humans [7]. Hordenine detectable in beer is produced during the malting process. The concentration in beer depends on the temperature, humidity, duration of the germination and the CO<sub>2</sub> concentration during the malting procedure [8]. In legal medicine hordenine can be used as a new marker for detection of consumption of beer [9-11].

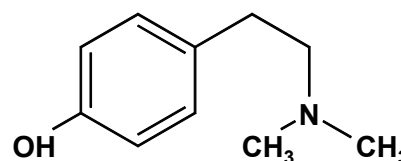


Fig. 1. Structure of hordenine.

## 2. Material and Methods

### 2.1. Sample Preparation

Add 20 µl beer to a vial, add 980 µl of a mixture of water/methanol (65:35, contained 0,1% formic acid) to beer sample, transfer 40 µl of this mixture to a sample vial, add 20 µl standard (hordenine-d4), inject 10 µl per analysis to LC-MS/MS.

### 2.2. MS-Parameter, Calibration and Detection Range

Waters Acquity Ultra performance LC-MS/MS; Column Agilent Eclipse XDB-C18, 5 µm, 4,6 x 150 mm); Solvent A: water (0,1% formic acid), Solvent B: methanol (0,1 % formic acid), flowrate: 0,4 ml/min, gradient: initial 65%A/35%B, 0.5 min 65%A/35%B, 10 min 15%A/85%B, 12.25 min 5%A/95%B, 12.50 min, 65%A/35%B, 16 min 65%A/35%B.

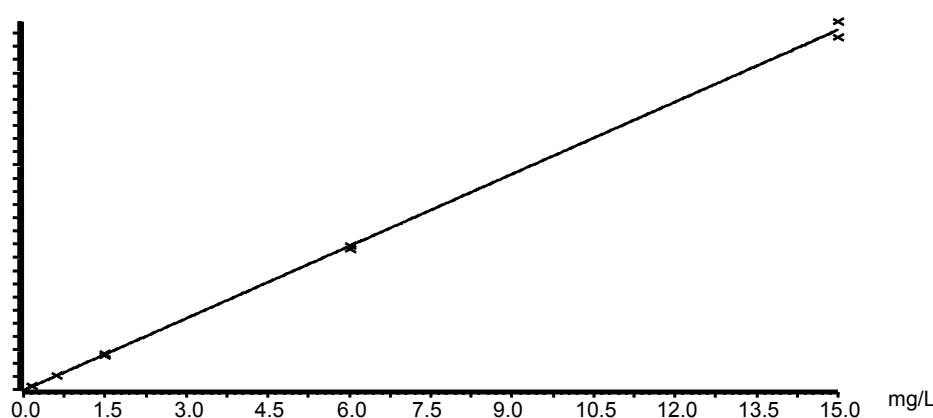


Fig. 2. Calibration curve of hordenine ( $r^2 = 0.9992$ ).

Tab. 1. Target, quantifier ions and detection range of hordenine.

| Substance    | Tgt. m/z | Q1 m/z | Q2 m/z | Range mg/L | LOQ mg/L | LOD mg/L |
|--------------|----------|--------|--------|------------|----------|----------|
| Hordenine    | 121      | 103    | 91     | 0.15-15    | 0.15     | 0.1      |
| Hordenine-d4 | 121      | 103    | 91     |            |          |          |

## 3. Results and Discussion

Table 2 lists the concentrations of hordenine in different types of beer and other beverages. In summary it can be concluded that:

- in Altbier/dark beer, Kölsch and Pils no significant differences in the average concentrations of hordenine are found.
- top- and bottom-fermenting beers do not differ each other in their hordenine concentrations.
- mixed beers show lower hordenine concentrations.
- hordenine was detected in alcohol-free beers too. The concentrations are lower (except malt beer) than in alcoholic beers.
- hordenine was not found in beverages with amounts of bitter orange such as Aperol®.
- orange juice contains traces of hordenine only.

Tab. 2. Hordenine concentrations (mg/L) in beer and other beverages.

| <b>Beer brand</b>            | <b>Hordenine<br/>[mg/L]</b> | <b>Beer brand</b>                | <b>Hordenine<br/>[mg/L]</b> |
|------------------------------|-----------------------------|----------------------------------|-----------------------------|
| <b>Strong beer (average)</b> | <b>5.16</b>                 | <b>alcohol-free (average)</b>    | <b>1.34</b>                 |
| Andechs Bergbock             | 4.70                        | Clausthaler (alcohol-free)       | 1.44                        |
| Andechs Doppelbock           | 6.15                        | Krombacher Radler (alcohol-free) | 1.35                        |
| Einbecker Urbock             | 4.38                        | Zötler Weizen (alcohol-free)     | 1.24                        |
| Paulaner Salvator            | 5.76                        | <b>mixed beer (average)</b>      | <b>1.74</b>                 |
| Mönchshof Bock               | 4.79                        | Brinkhoffs No1 (Radler)          | 2.19                        |
| <b>Pils (average)</b>        | <b>2.70</b>                 | Mixery                           | 1.82                        |
| Astra Urtyp                  | 2.23                        | Veltins Radler                   | 1.56                        |
| Becks                        | 3.08                        | <b>wheat beer (average)</b>      | <b>1.81</b>                 |
| Bitburger Pils               | 3.65                        | Erdinger Weißbier                | 1.84                        |
| Breznak Böhmisch             | 2.55                        | Hofbräu                          | 1.62                        |
| Dortmunder Export            | 2.72                        | Zötler Hefe                      | 1.97                        |
| Flensburger Pilsener         | 2.43                        | <b>other beer (average)</b>      | <b>1.32</b>                 |
| Karlsberg Pils               | 3.09                        | Beer Premium Lager               | 0.64                        |
| Königspilsener               | 3.18                        | Corona Extra                     | 1.29                        |
| Oscar Maxxum                 | 2.26                        | Desperados                       | 1.75                        |
| Radeberger                   | 2.54                        | Germania Edelpils                | 0.75                        |
| Veltins                      | 2.89                        | Guinness                         | 0.90                        |
| Warsteiner                   | 2.42                        | Kilkenny                         | 1.66                        |
| Zywiec Pils                  | 2.52                        | Rhenania alt                     | 1.70                        |
| <b>Alt (average)</b>         | <b>2.80</b>                 | Salitos                          | 1.80                        |
| Boltens Ur-Alt               | 3.73                        | Warka Pils                       | 1.43                        |
| Diebels Alt                  | 2.41                        | <b>other drinks average)</b>     |                             |
| Frankenheim Alt              | 2.84                        | Aperol                           | not detectable              |
| Füchschen Alt                | 2.17                        | Bionade                          | not detectable              |
| Schlösser Alt                | 3.12                        | Wine                             | not detectable              |
| Uerige Alt                   | 2.55                        | Orange juice                     | traces                      |
| <b>Kölsch (average)</b>      | <b>2.97</b>                 |                                  |                             |
| Dom Kölsch                   | 2.48                        |                                  |                             |
| Früh Kölsch                  | 3.54                        |                                  |                             |
| Mühlen Kölsch                | 3.83                        |                                  |                             |
| Reiðdorf Kölsch              | 3.26                        |                                  |                             |

We conclude: The concentration of hordenine in beer depends on the malting process. The subsequent brewing process has only a minor influence on the hordenine concentration. That is the reason why the hordenine concentrations of top- and bottom-fermenting beers are nearly the same. Increasing amounts of hordenine in strong beers is the effect of a higher mash proportion in this type of beer. Hordenine could be reliably detected in all examined types of beer. It is found in blood and urine and is a new marker of beer consumption [9-11].

#### 4. References

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