Hopsteiner

Hop Analysis: International cooperation and new challenges by craft beer

Martin Biendl

Hop Analysis - International Cooperation

Some of the hop methods in „ASBC Methods of Analysis“ and “EBC-Analytica“ are identical

Example: ASBC Hops 14 = EBC 7.7

α-Acids and β-Acids in Hops and Hop Extracts by HPLC (International Method)

Status International: also recommended by BCOJ

Hop Analysis - International Cooperation

All calibration standards recommended by ASBC, EBC and BCOJ for HPLC analysis of hop bitter compounds are identical

Basis: Foundation of the “Joint EBC/ASBC Hop Standard Subcommittee“ in 1994 with introduction of “ICE-1“ (the first “International Calibration Extract“ for alpha- and beta-acids)

1998: Foundation of the “International Subcommittee for Isomerized Hop Alpha-Acids“ (ASBC, EBC, IOB, BCOJ)

International Subcommittee for Isomerized Hop Alpha-Acids - Mission

Release of calibration standards for HPLC analysis of iso-alpha- and all kinds of reduced iso-alpha-acids in hop products or beer

Introduction of ICS (International Calibration Standard) for

- Iso-alpha-acids (ICS-I1)*
- Rho-iso-alpha-acids (ICS-R1)*
- Tetrahydro-iso-alpha-acids (ICS-T1)
- Hexahydro-iso-alpha-acids (ICS-H1)*

* Complexes with Dicyclohexylamine (DCHA)

Production procedures: *Maye et al., 1999; Thornton et al., 1993*

International Subcommittee for Isomerized Hop Alpha-Acids - Structure

Two chairs: *Bob Foster* (ASBC) / *Martin Biendl* (EBC)

Secretary: *John Paul Maye* (ASBC)

Around 10 active members from ASBC, EBC and BCOJ

Regular annual meeting in conjunction with ASBC conferences

New name since 2010:

International Hop Standards Committee (ASBC/EBC/BCOJ)

International Hop Standards Committee (ASBC/EBC/BCOJ) – Member tasks

Production of suitable standard batches followed by purity determinations (solvent residues, elemental analysis, etc.)

Participation in international collaborative trials to confirm the purity of a new standard batch and to assess its exact values

Producer of the standard batch fills small vials (à 40 g ICE or 250 mg ICS) and supplies them to ASBC and EBC for sale

Announcement of a new standard via press release

International Hop Standards Committee (ASBC/EBC/BCOJ) – Current activities

New batch of ICS-H2 just released

Collaborative trial on a new batch of ICS-R3 just finished

Production of new batches for ICS-I4 and ICE-4 will start soon

Plan to extend the range of standards for dry hopped beers

Hop Analysis - New challenges by craft beer

Composition of a regular Pilsener (mg/l):

Beer	Iso-alpha-acids	Alpha-acids	Beta-acids	Humulinones	Hulupones
Pilsener	30.1	0.8	< 0.3	0.5	0.2

Bitterness level mainly (only) determined by iso-alpha-acids

Bitterness quality positively influenced by minor constituents

(*Intelmann et al., 2009; Haseleu et al., 2009; Dresel et al., 2015*)

Hop Analysis - New challenges by craft beer

Composition of the same Pilsener, additionally dry hopped (mg/l):

Beer	Iso-alpha-acids	Alpha-acids	Beta-acids	Humulinones	Hulupones
Pilsener	30.1	0.8	< 0.3	0.5	0.2
Pilsener, dry hopped	25.9	5.6	0.3	7.6	2.5

Bitterness level of dry hopped beers not only determined by iso-alpha-acids but also by alpha-acids, humulinones,hulupones

(Maye et al., 2016; Algazzali and Shellhammer, 2016)

Hop Analysis - New challenges by craft beer

International Hop Standards Committee (ASBC/EBC/BCOJ) is considering the production of batches for the release of a

Humulinone standard (DCHA-Humulinone)*

Hulupone standard (DCHA-Hulupone)*

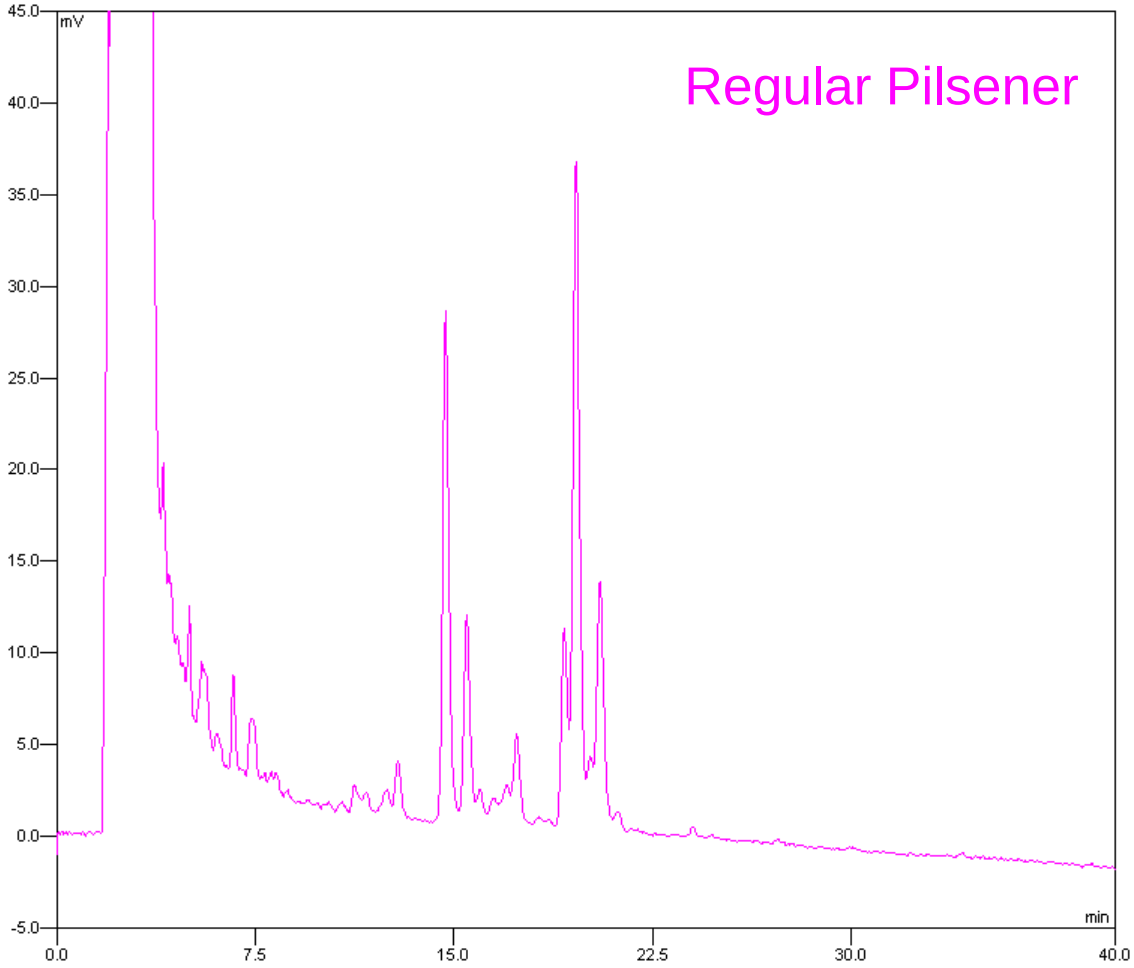
Alpha-acids standard (DCHA-Alpha)**

Beta-acids standard (DCHA-Beta)**

According production processes and stability data published by

* *Maye et al., 2016* and ***Maye and Leker, 2014*

Analytica-EBC - Method 9.47: Determination of iso-alpha- and reduced iso-alpha-acids in beer

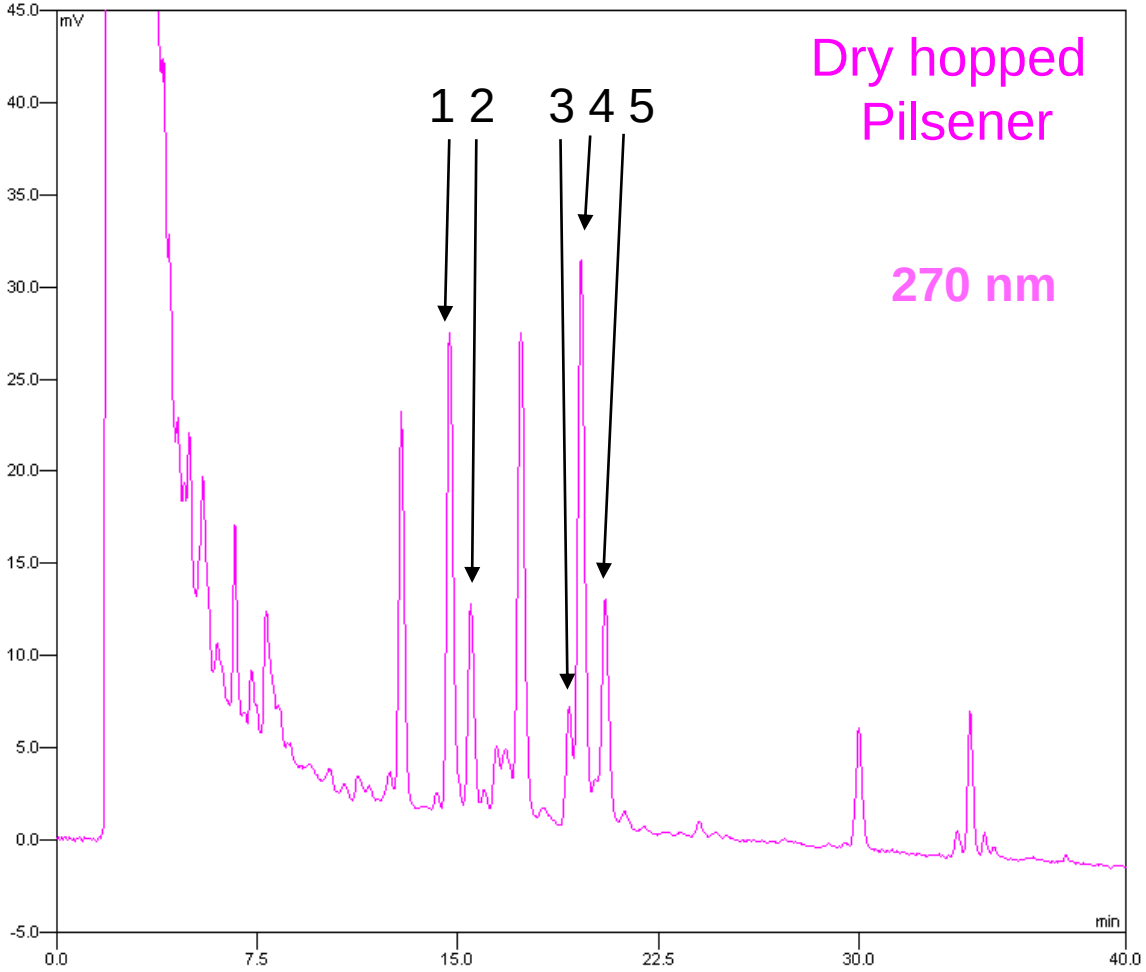


Beer sample preparation:
Dilution with methanol (1:1)
Direct injection into HPLC after separation of the precipitate

HPLC conditions:
Column: Zorbax Eclipse C8
Detection: UV (270 nm)
Eluent A: Methanol
Eluent B: 30 % Acetonitrile / 70 % Citric acid (1%)

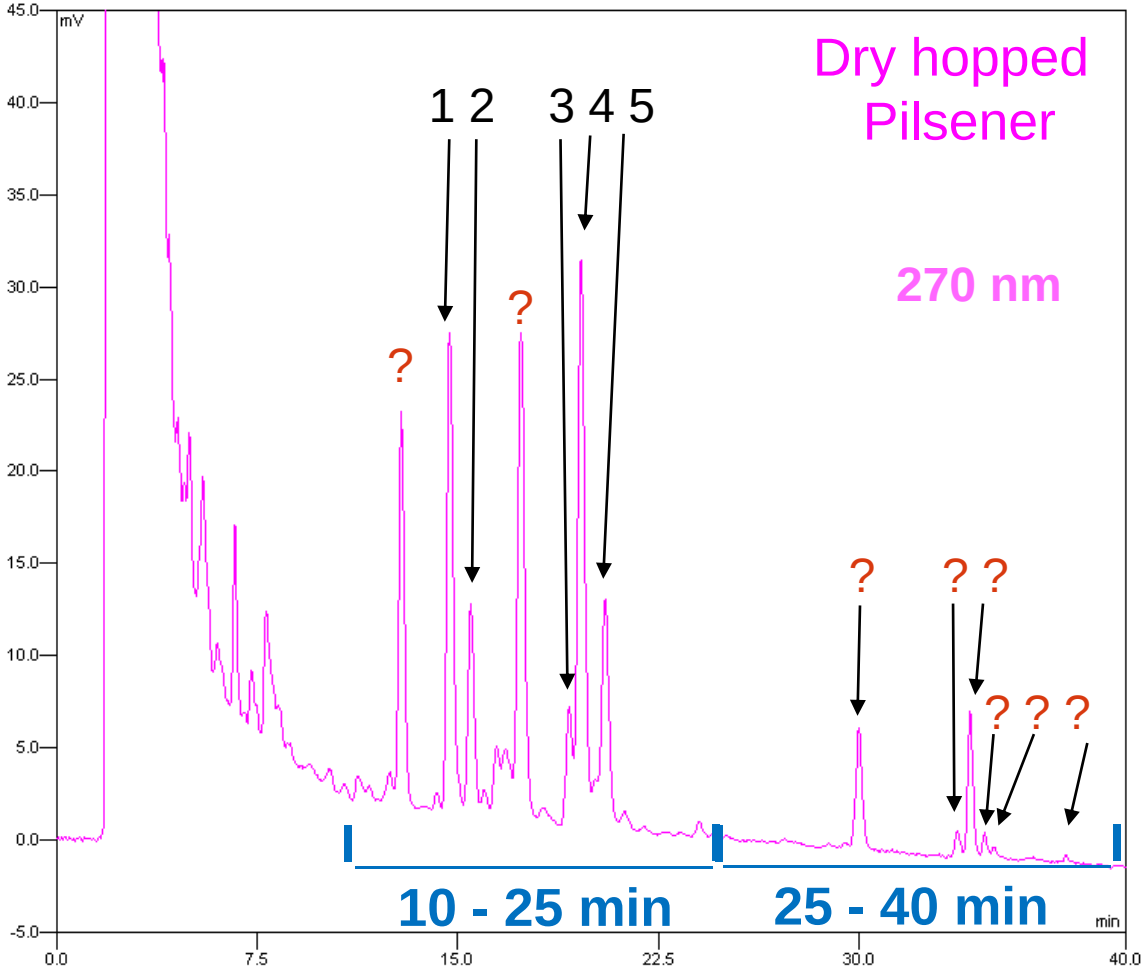
HPLC programme:
0-5 min: 85 % Solvent B
5-45 min: 85-20 % Solvent B
45-50 min: 20-85 % Solvent B
50-60 min: 85 % Solvent B

Dry hopped beer according to method EBC 9.47



1: cis-co-isohumulone
2: trans-co-isohumulone
3: cis-n-isohumulone
4: trans-n-isohumulone
5: cis/trans-ad-isohumulone

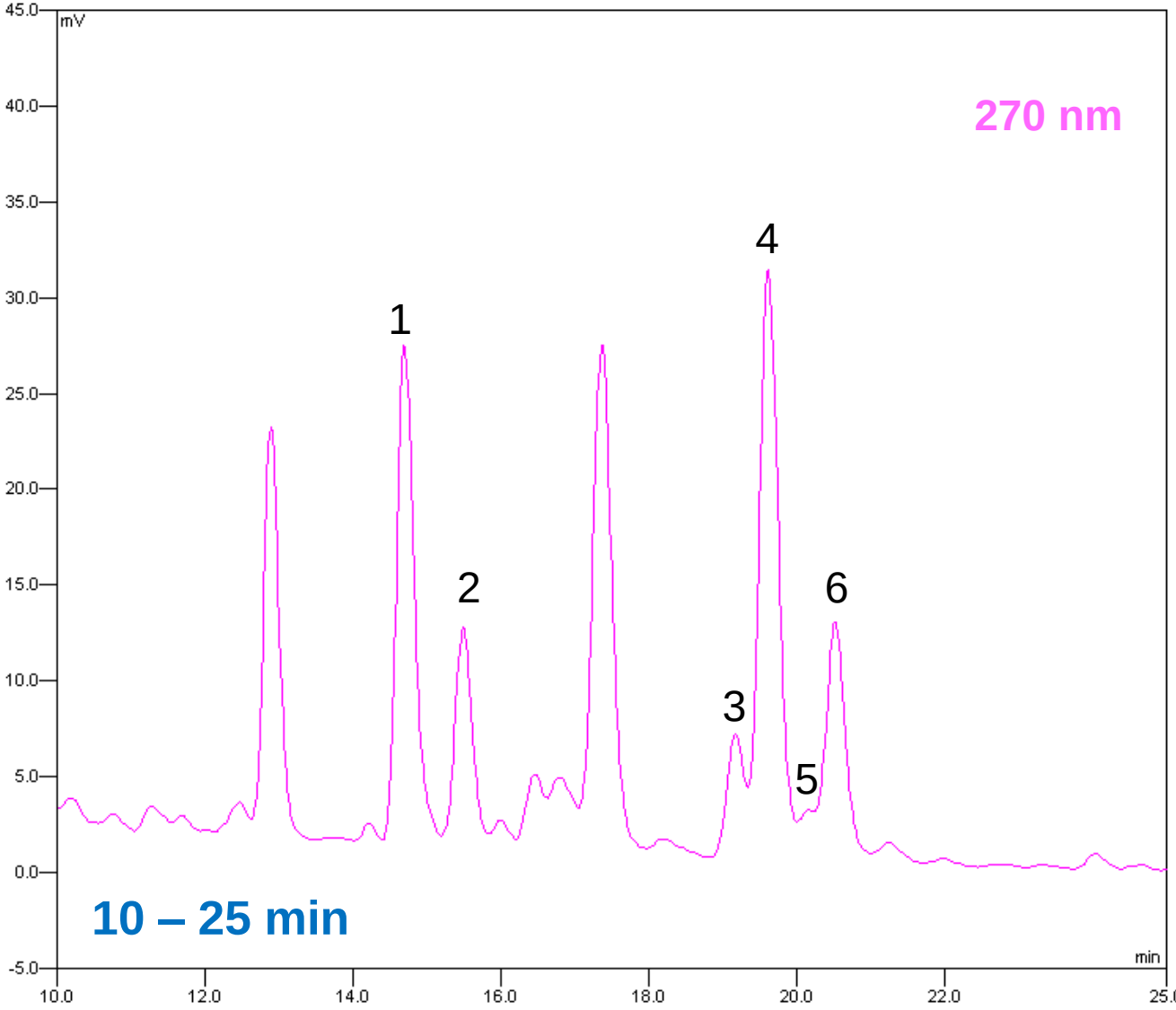
Dry hopped beer according to method EBC 9.47



1: cis-co-isohumulone
2: trans-co-isohumulone
3: cis-n-isohumulone
4: trans-n-isohumulone
5: cis/trans-ad-isohumulone

? alpha-acids (humulones)
? beta-acids (lupulons)
? humulinones
? hulupones

10-25 min / 270 nm: Dry hopped beer



1: cis-co-isohumulone

2: trans-co-isohumulone

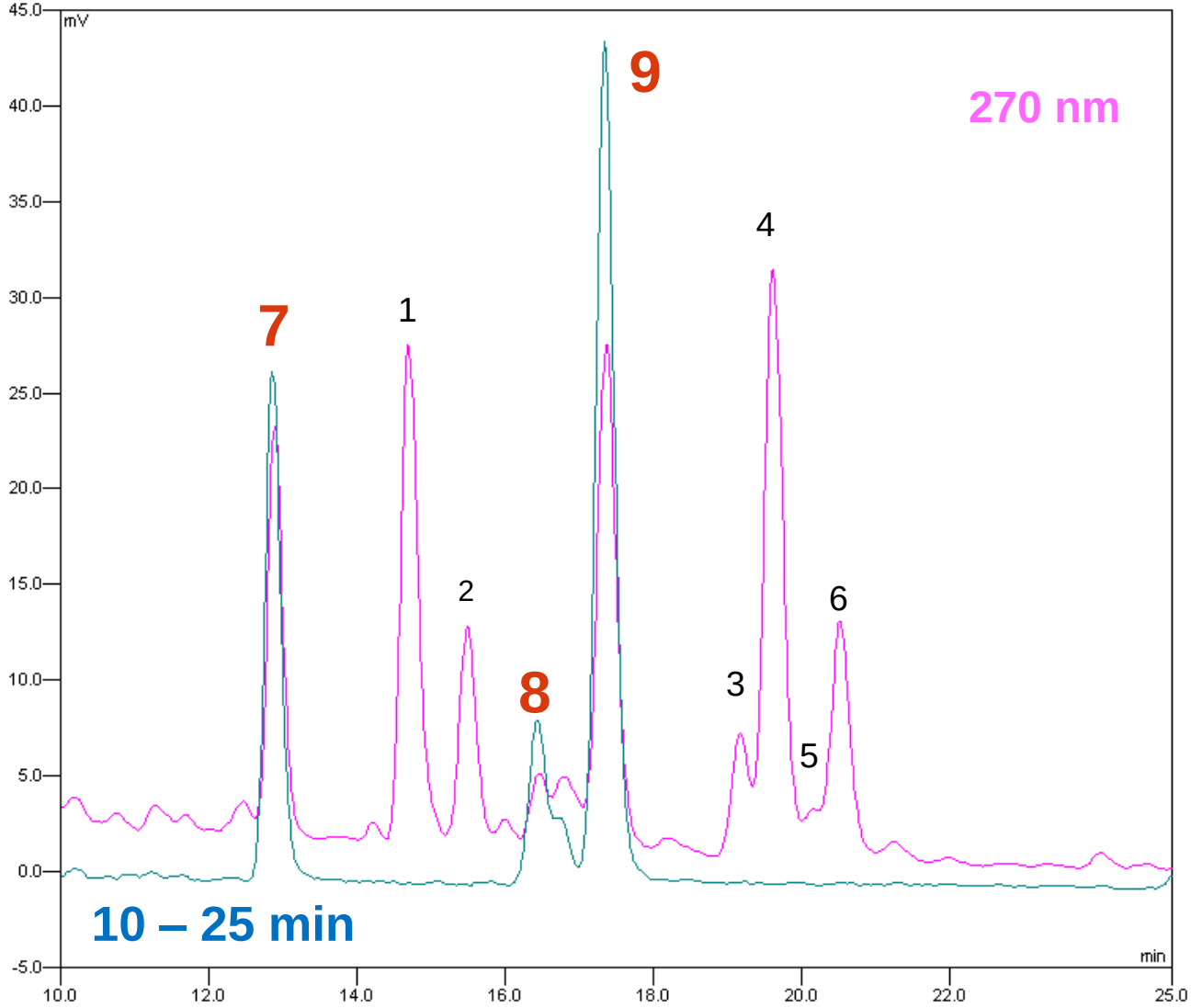
3: cis-ad-isohumulone

4: cis-n-isohumulone

5: trans-ad-isohumulone

6: trans-n-isohumulone

Dry hopped beer + DCHA-Humulinone



1: cis-co-isohumulone

2: trans-co-isohumulone

3: cis-ad-isohumulone

4: cis-n-isohumulone

5: trans-ad-isohumulone

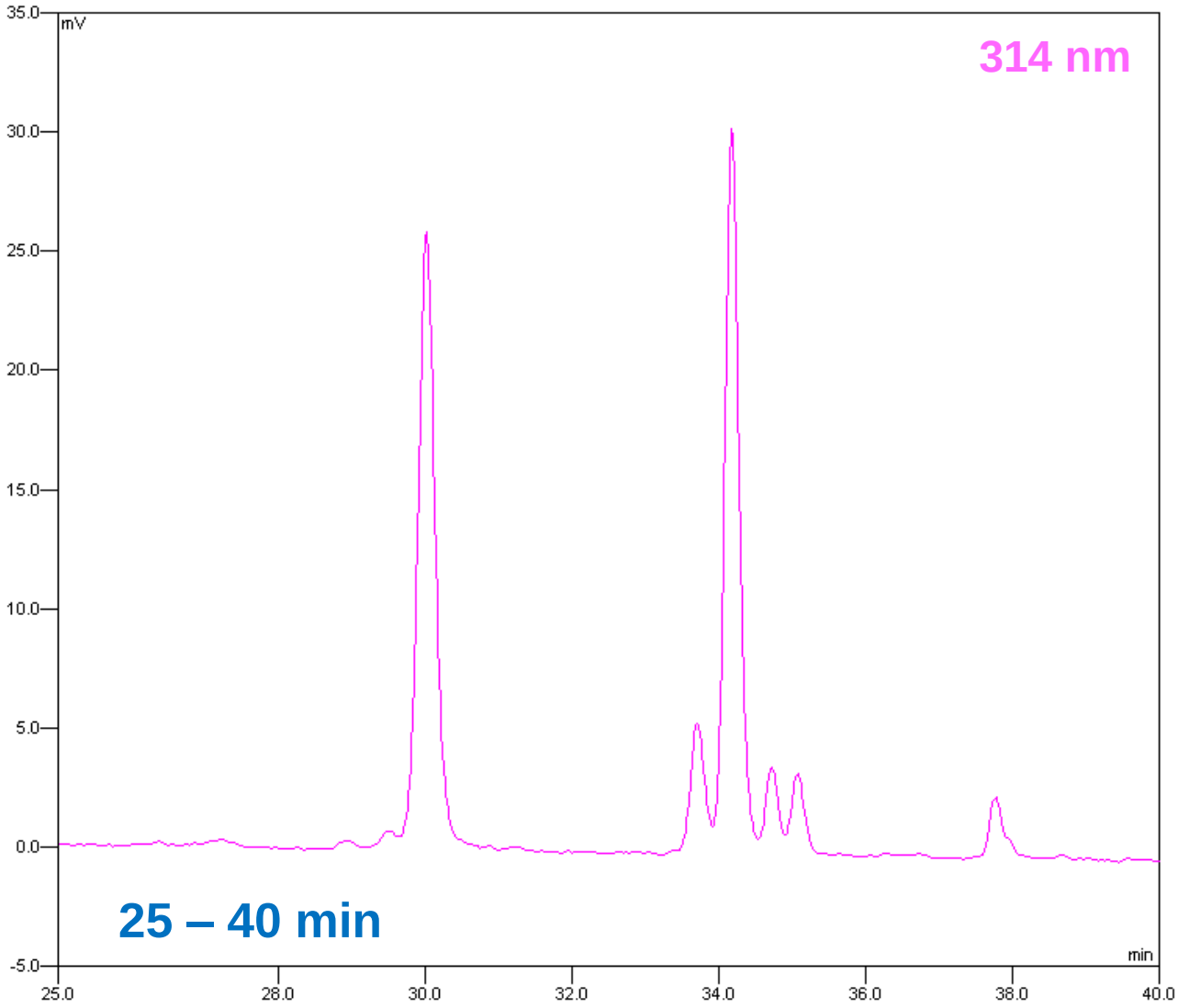
6: trans-n-isohumulone

7: co-humulinone

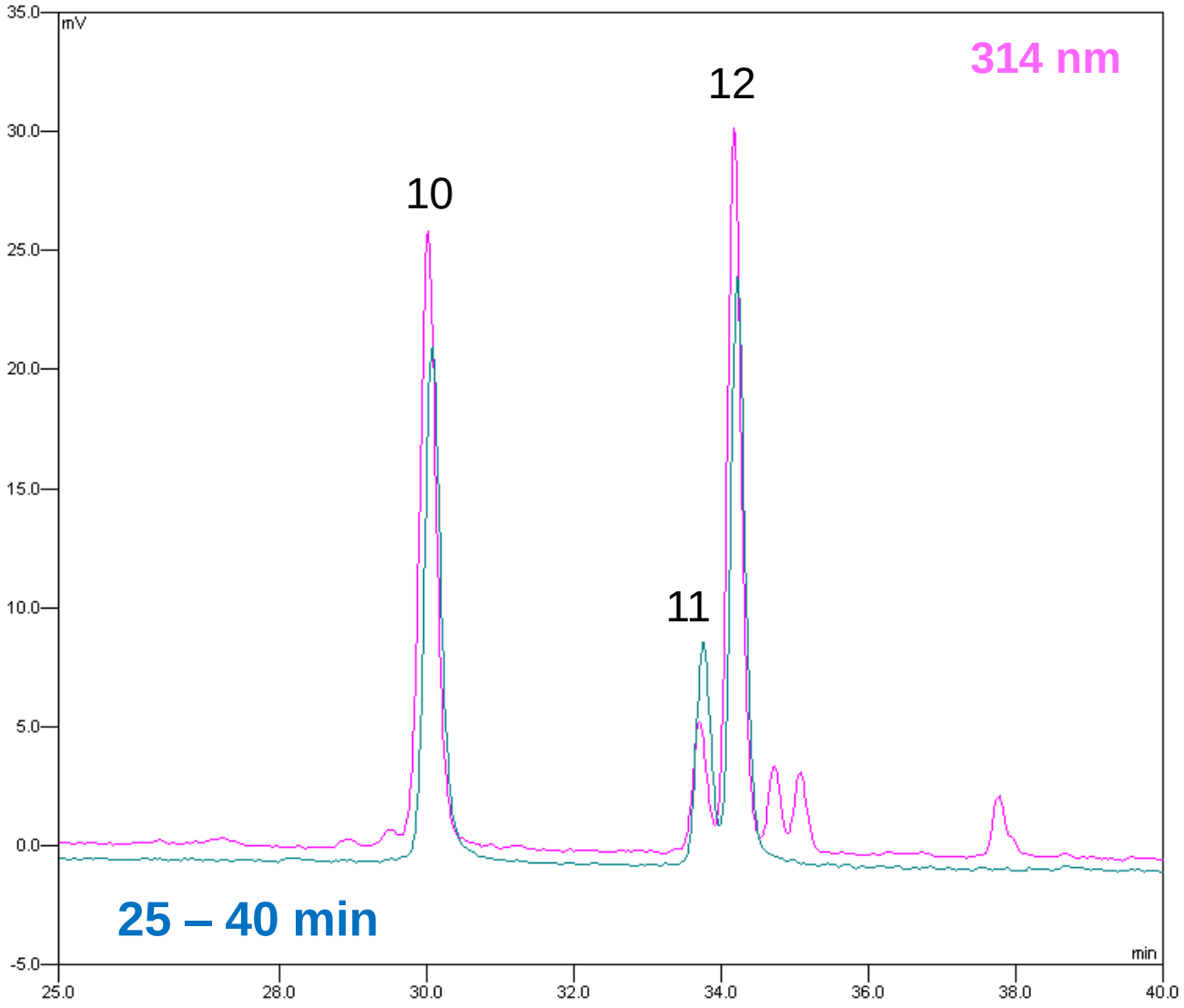
8: ad-humulinone

9: n-humulinone

25-40 min / 314 nm: Dry hopped beer



Dry hopped beer + DCHA-Humulone

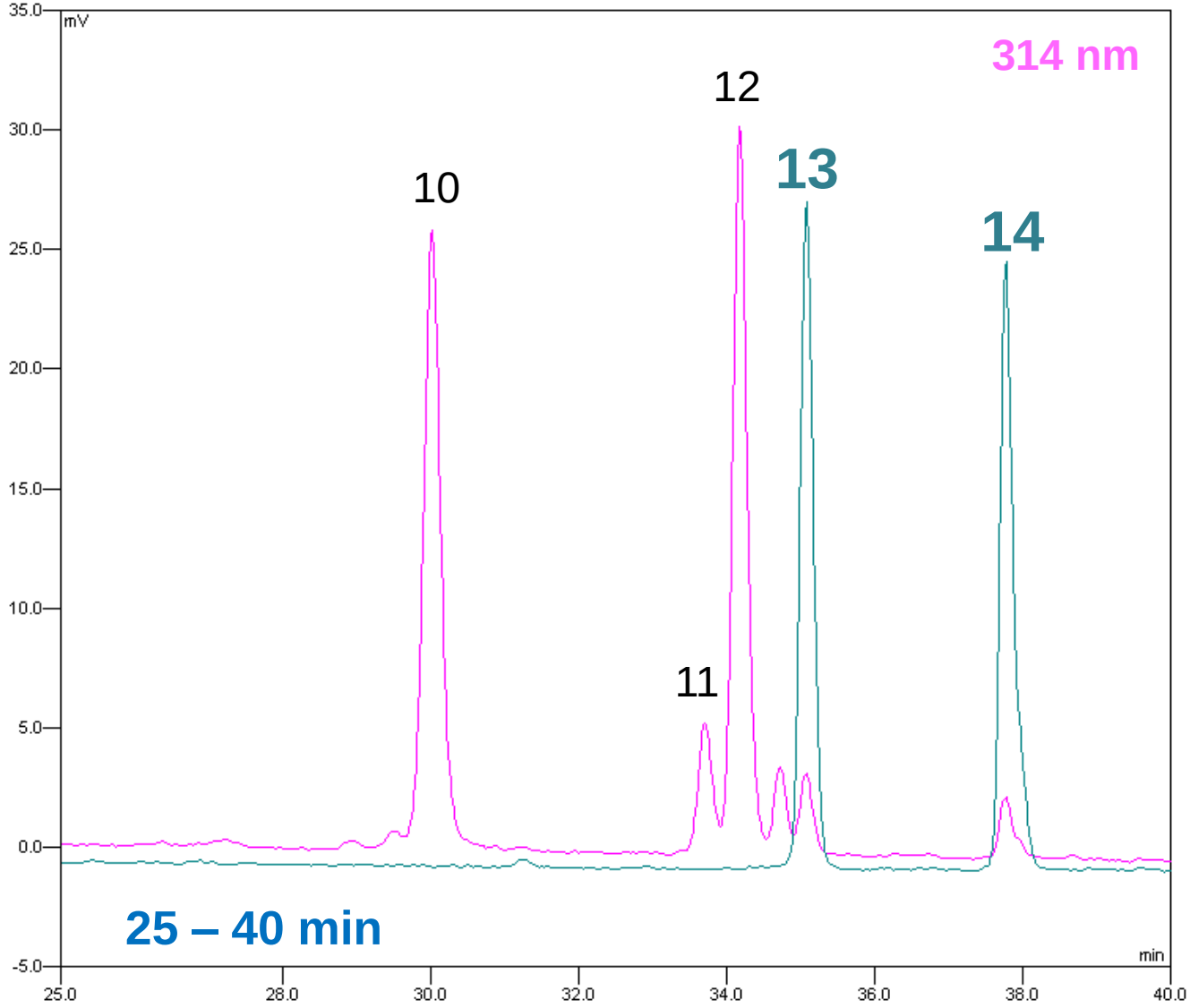


10: co-humulone

11: ad-humulone

12: n-humulone

Dry hopped beer + DCHA-Lupulone



10: co-humulone

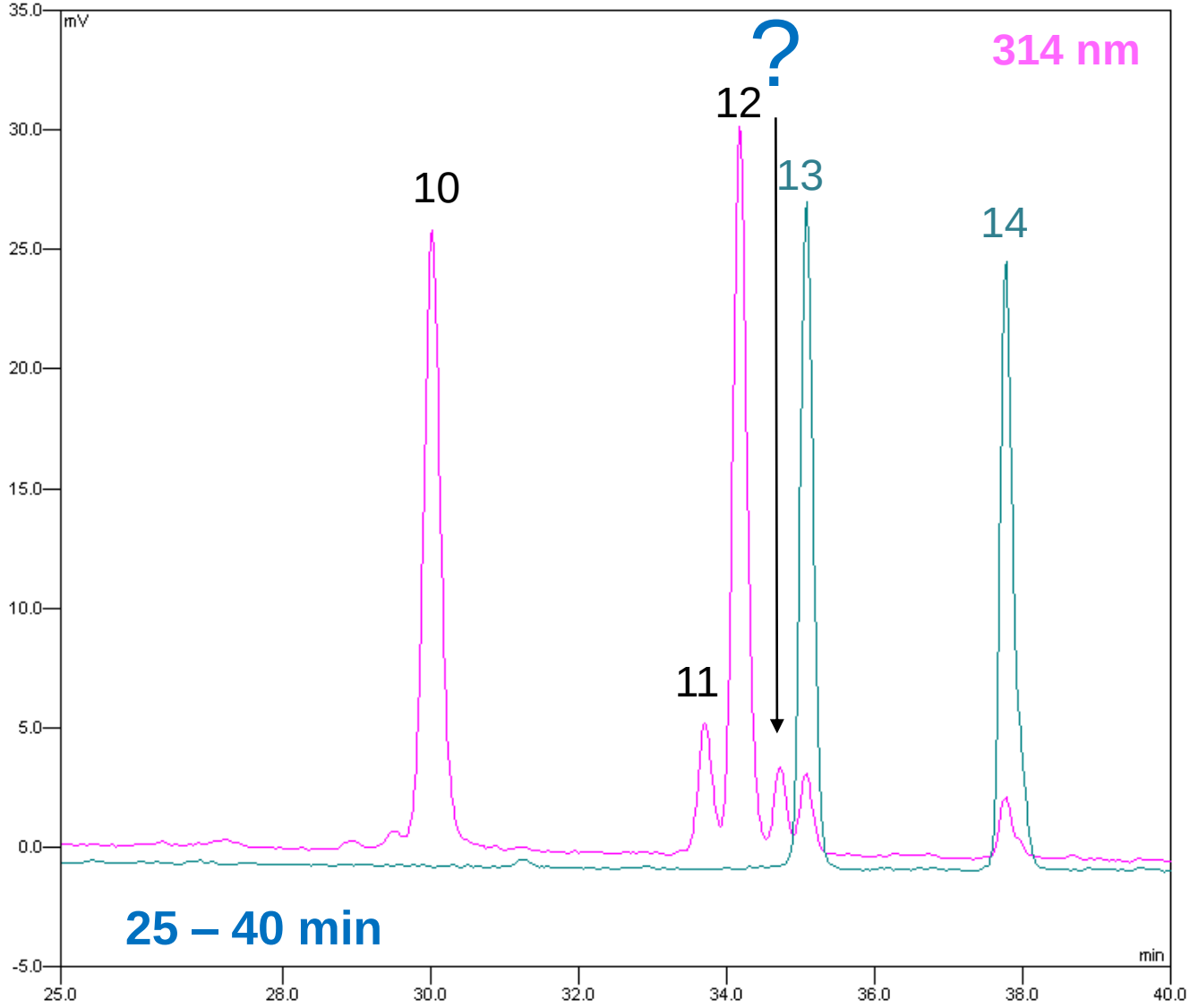
11: ad-humulone

12: n-humulone

13: co-lupulone

14: n/ad-lupulone

Dry hopped beer + DCHA-Lupulone



10: co-humulone

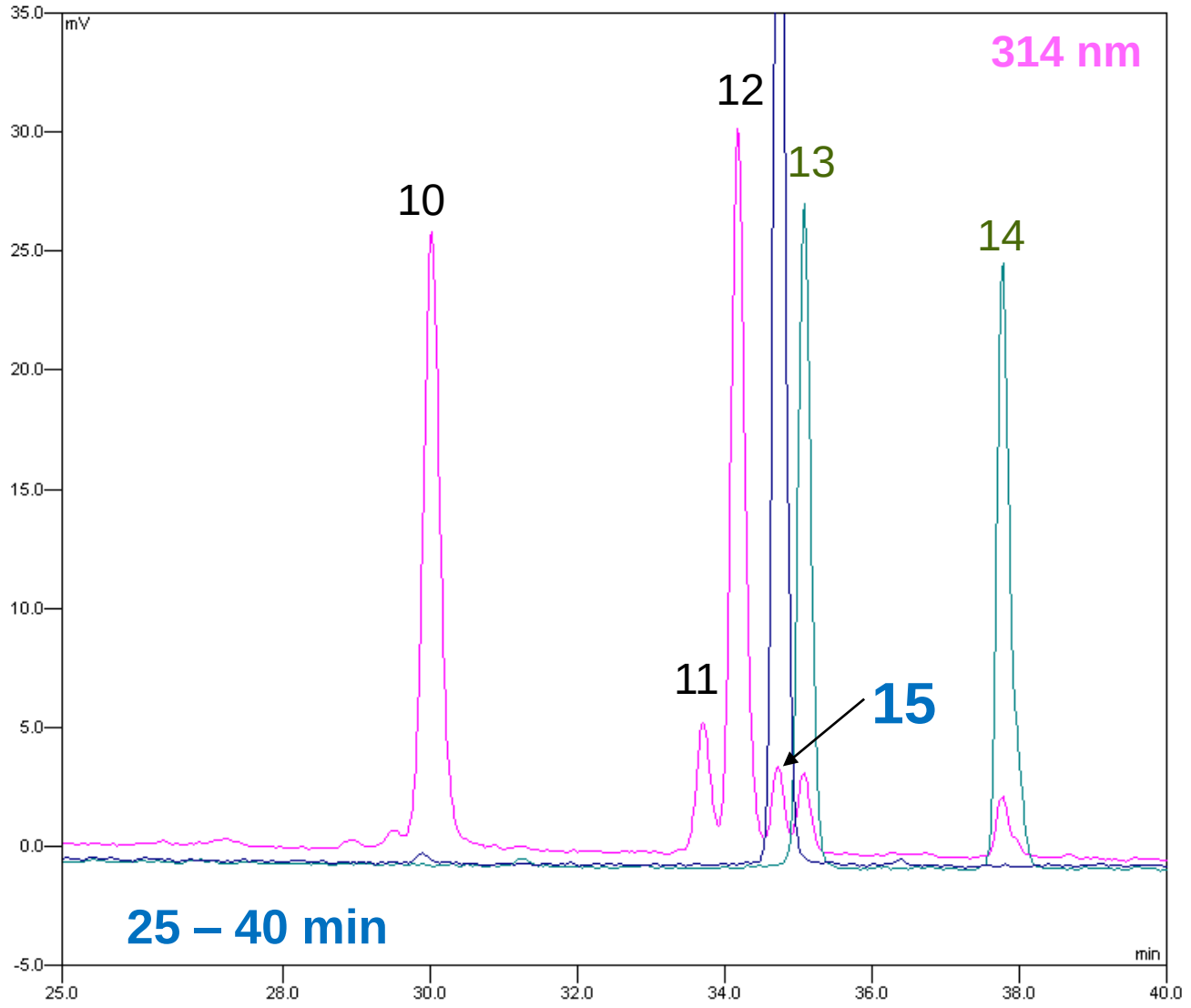
11: ad-humulone

12: n-humulone

13: co-lupulone

14: n/ad-lupulone

Beer + DCHA-Lupulone + Xanthohumol



9: co-humulone

10: ad-humulone

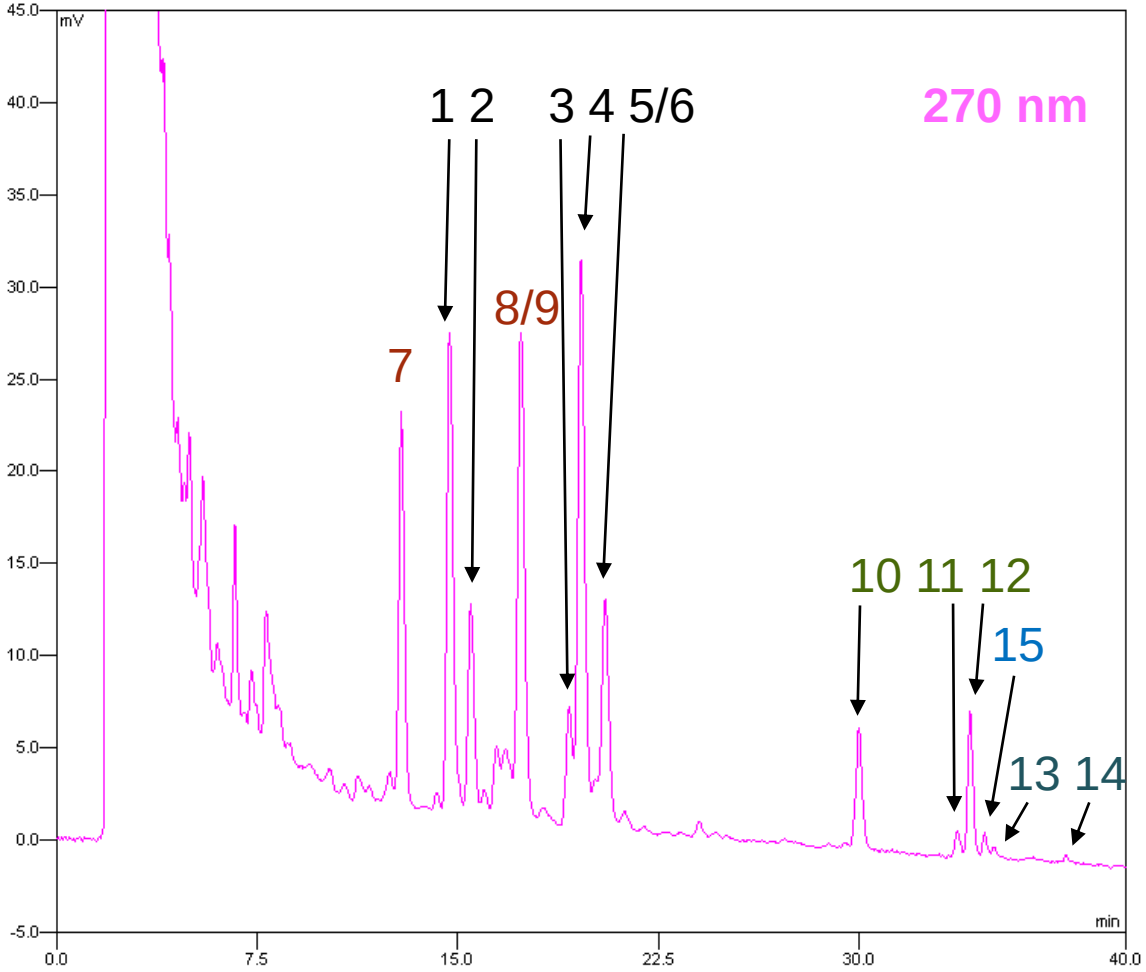
11: n-humulone

13: co-lupulone

14: n/ad-lupulone

15: xanthohumol

Dry hopped beer according to method EBC 9.47



1: cis-co-isohumulone
2: trans-co-isohumulone
3: cis-n-isohumulone
4: trans-n-isohumulone
5/6: cis/trans-ad-isohumul.

7: co-humulinone
8/9: n/ad-humulinone

10: co-humulone
11: ad-humulone
12: n-humulone

13: co-lupulone
14: n/ad-lupulone

15: xanthohumol

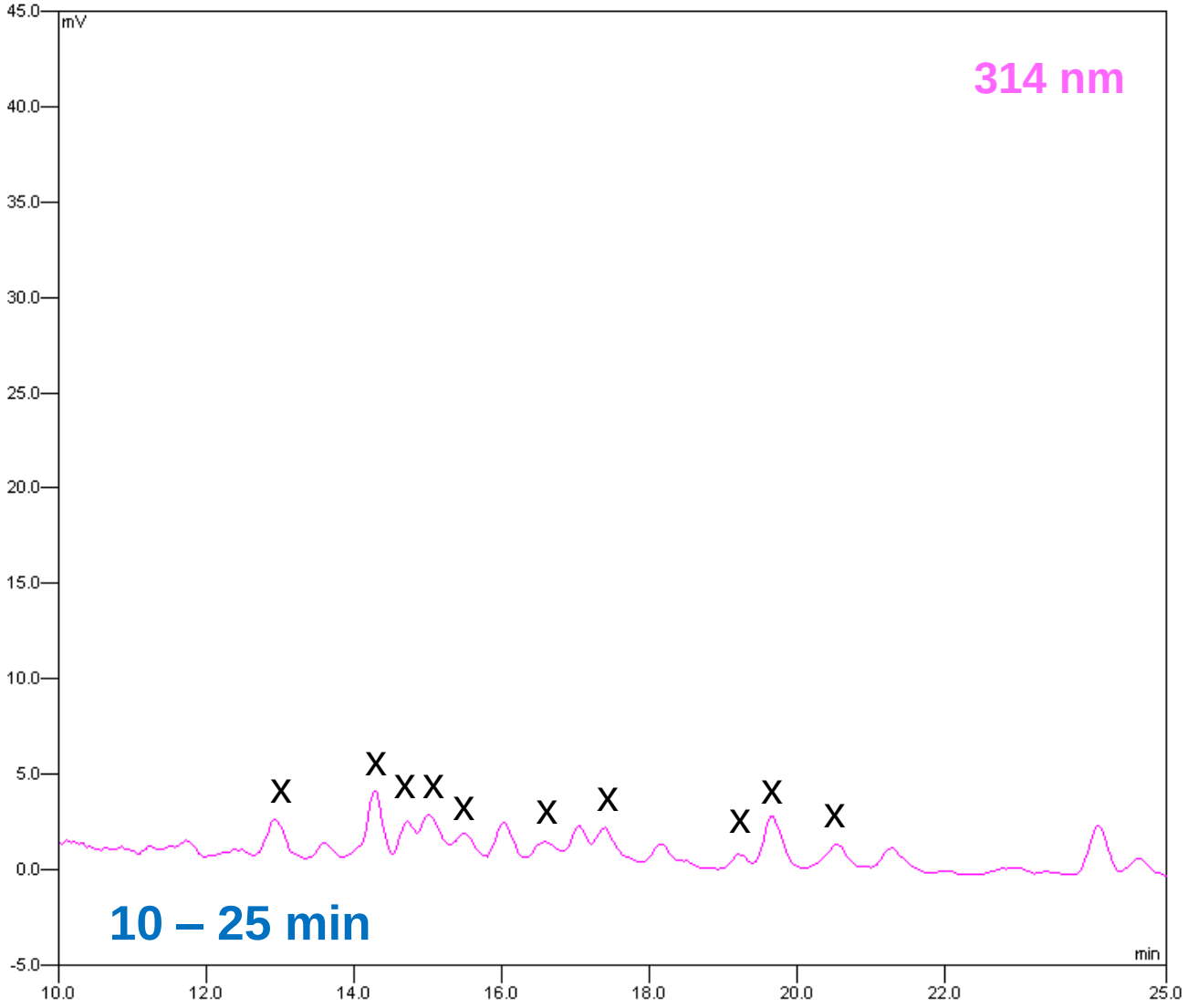
Composition of different dry hopped beers (mg/l) Method EBC 9.47

Beer	Iso-Alpha	Alpha	Beta	Humu-linone	Hulu-pone	Xantho-humol	Isoxantho-humol
1*	20.1	15.7	1.7	10.8	1.6	0.8	0.4
2**	12.5	23.2	3.4	7.9	2.2	8.6	1.2

*1: Ale, dry hopped

**2: Stout, dry hopped

10-25 min/314 nm: Dry hopped beer

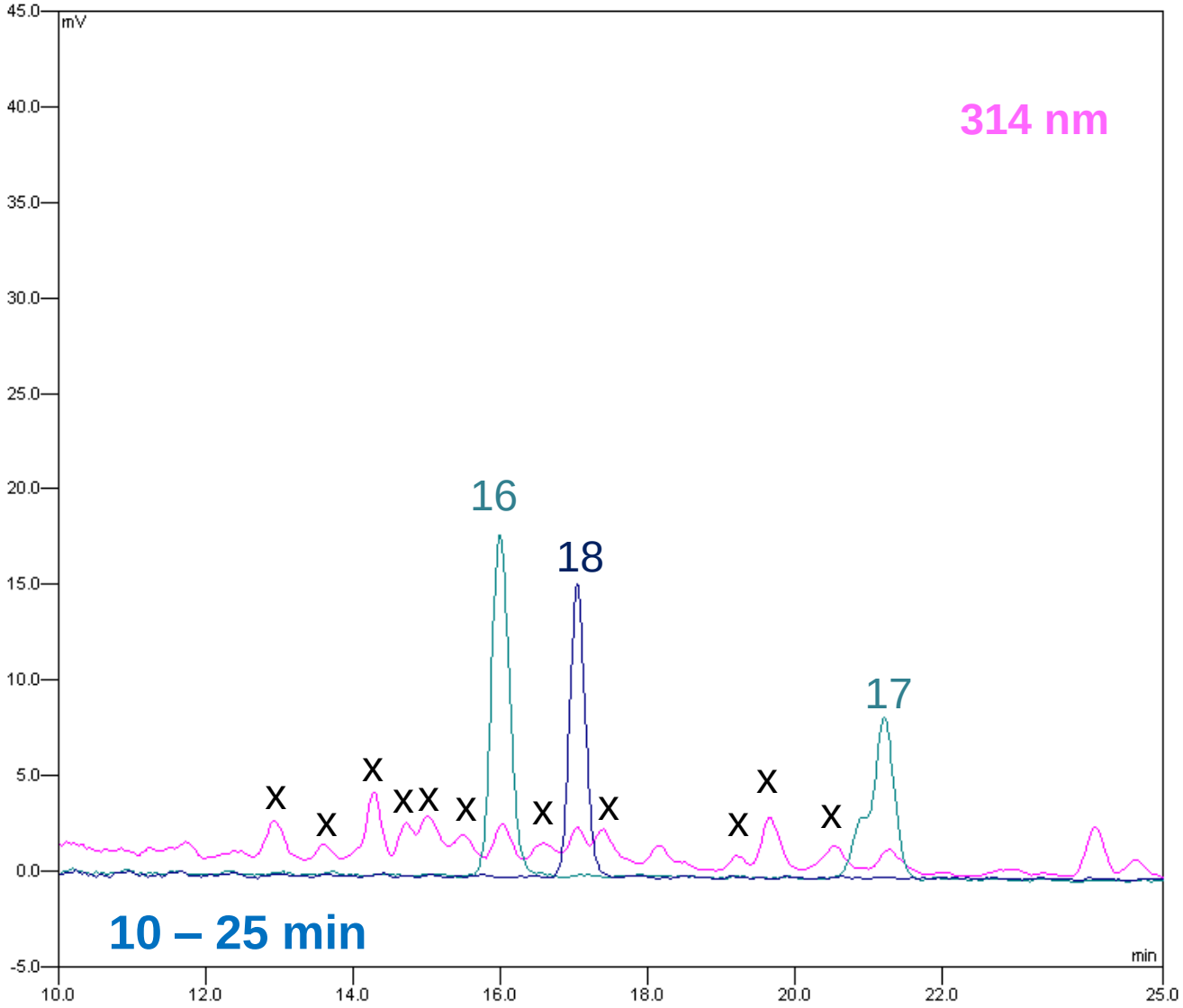


x x x:

cis/trans-co/n/ad-isohumulones

co/n/ad-humulinones

Beer + DCHA-Hulupone + Isoxanthohumol



x x x:

cis/trans-co/n/ad-isohumulones

co/n/ad-humulinones

16: co-hulupone

17: ad/n-hulupone

18: isoxanthohumol

International collaborative trial for analysis of dry hopped beer according to method EBC 9.47

Organisation by hop section of EBC AC: end of 2016 (plan)

Samples: 4 dry hopped beers with different compound levels

Parameters:

Iso-alpha-acids (isohumulones), alpha-acids (humulones), humulinones, hulupones, beta-acids (lupulones)

According calibration standards (DCHA complexes) supplied

In case of interest in participitation: mbiendl@hopsteiner.de

New challenges by craft beer – international method for analysis of hop aroma compounds?

Aroma compounds are only present in ppb levels (or lower) – gaschromatography-mass-spectrometry equipment is essential

Many different methods for sample preparation are published (solid-phase micro-extraction, purge-and-trap, headspace-trap, stir-bar sorptive extraction) – which can be recommended?

So far only labeled calibration standards can be recommended

Which compounds are relevant for dry hopping aroma?

New challenges by craft beer – analysis of various dry hopped beers on hop aroma compounds

Significant differences	Minor differences	Not detectable
linalool*	2-nonanone	e,z-1,3,5-undecatriene
geraniol*	2-decanone	α- and β-pinene
citronellol	2-undecanone	propyl-2-methylbutanoate
terpineol	2-dodecanone	methyl-2-methylbutanoate
myrcene*	2-tridecanone	ethylisobutanoate
α-humulene	β-damascenone	ethyl-2-methylbutanoate
β-caryophyllene	β-limonene	methylhexanoate
β-farnesene	methylnonanoate	methylheptanoate
2-methylbutylisobutanoate*	methyldecanoate	methyloctanoate
isobutylisobutanoate	ethyldodecanoate	3-methylbutylpropanoate

* Key aroma compounds (*Fritsch et al., 2005; Takoi et al., 2009*)

Hop Analysis - New challenges by craft beer

Key hop aroma compounds in different beers (ppb):

Beer	Myrcene	Linalool	Geraniol	2-Methylbutyl-isobutanoate
Regular Pale Ale	15	20	n.d.*	< 5

n.d. = not detected

Hop Analysis - New challenges by craft beer

Key hop aroma compounds in different beers (ppb):

Beer	Myrcene	Linalool	Geraniol	2-Methylbutyl-isobutanoate
Regular Pale Ale	15	20	n.d.	< 5
Dry hopped 300 g/hl	293	329	56	73

Significant increase of key aroma compounds after dry hopping

Hop Analysis - New challenges by craft beer

Key hop aroma compounds in different beers (ppb):

Beer	Myrcene	Linalool	Geraniol	2-Methylbutyl-isobutanoate
Regular Pale Ale	15	20	n.d.	< 5
Dry hopped 300 g/hl	293	329	56	73
Dry hopped 500 g/hl	513	485	77	109

Increase is dependent on amount of hops used for dry hopping

Our equipment for hop aroma analysis of beer

Focus GC with DSQ II Mass Spectrometer (Thermo Scientific)

Turbo Matrix™ Headspace-Trap Sampler (Perkin Elmer)

International collaborative trial for analysis of dry hopped beer by headspace-trap GC-MS?

Our method is published (*Schmidt and Biendl, 2016*)

There are more than 10 brewery labs using this equipment

Kick-off meeting today afternoon 5:00 - 6:00pm:

Sheraton Downtown Denver, Directors Row E

- Background on HS technology and methods presented
- Experiences using HS-GC/MS
- Future design considerations