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Single-run capillary electrochromatographic analysis of hop acids and prenylated hop flavonoids

Key Words: Capillary electrochromatography; Hop acids; Prenylated flavonoids; Polyphenols

1 Introduction

The characteristic bitter taste of pale beers is due to the presence of hop-derived substances, the so-called iso- α -acids, which are formed from the hop α -acids during the boiling of sweet wort with hops (*Humulus lupulus* L.) in the brewery [1]. The analysis of hop acids, which include, in addition to α -acids, also β -acids (**Figure 1**) is important to assess the quality of hops and hop products. Often, prenylated flavonoids interfere with the separation of α - and β -acids. The interest in prenylated flavonoids pertains to their bioactivities and a particular constituent, 8-prenylnaringenin, has been identified as the most potent phytoestrogen known to date [2, 3].

Previously, hop acids have been analysed by capillary zone electrophoresis (CZE), resulting in group separation of α - and β -acids, while individual components were not resolved [4]. Fast and efficient separation was achieved by micellar electrokinetic chromatography (MEKC), but the repeatability of the separation of the critical pair lupulone/adlupulone was poor due to the narrow pH-interval [4]. Microemulsion electrokinetic chromatography (MEEKC) proved to be a more rugged technique, allowing the use of a more extended pH range [5]. This method, developed for the separation of hop acids, was modified for a simultaneous analysis of hop acids and prenylated hop flavonoids. Again, the pH of the electrolyte was of utmost importance and slight pH-changes affected the separation dramatically [6].

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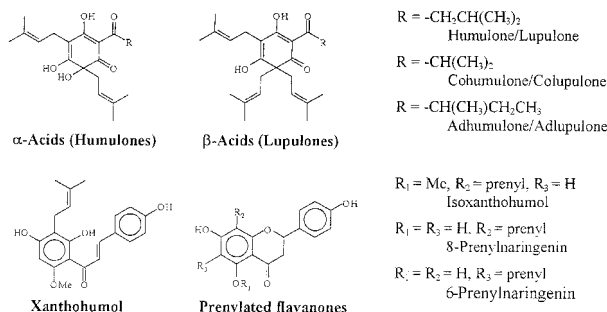


Figure 1. Structures of hop acids (humulones and lupulones) and of important prenylated hop flavonoids.

Application of capillary electrochromatography (CEC) reduces the effects of slight pH variations, thus affording reproducible results. Moreover, separation of the isomeric pairs humulone/adhumulone and lupulone/adlupulone improved greatly when compared to HPLC-analysis.

2 Materials and methods

2.1 Chemicals

Acetonitrile was purchased from Biosolve (Valkenswaard, The Netherlands). Formic acid and Tris (Tris(hydroxymethyl)aminomethane) were obtained from Merck-Belgrolabo (Overijse, Belgium). Water was of Milli-Q quality (Millipore, Brussels, Belgium).

2.2 Samples

Samples of the cones of the hop variety Hallertau Magnum (HM) were a gift from Dr. A. Forster from HVG, Barth, Raiser & Co., Wolnzach, Germany (now NATECO2 &

Co.). The samples were prepared by consecutive extraction of the hop cones (5 g) with petroleum ether (50 mL, 3 ×) and *n*-hexane (50 mL, 3 ×) to remove apolar material, and with methanol/water 3/1 (v/v) (80 mL, 3 ×) to isolate the hop acids and hop flavonoids. The methanolic fractions were collected, concentrated *in vacuo* and redissolved in 100 mL methanol/water 3/1 (v/v). Liquid-liquid extraction with petroleum ether (50 mL, 2 ×) and *n*-hexane (50 mL, 2 ×) removed any remaining apolar material. The residue, after removal of the solvent *in vacuo*, was redissolved in 100 mL methanol/water 1/1 (v/v).

Solid phase extraction (SPE) was applied using fivefold (water) diluted extracts. Bond Elut C18 cartridges containing 500 mg packing material (Varian, Zaventem, Belgium) were conditioned with 5 mL methanol and 5 mL methanol/water 1/9 (v/v) prior to application of the sample. Next, the cartridge was rinsed with 5 mL water and the compounds were eluted with 5 mL methanol. After removal of methanol, the residue was redissolved in 2 mL methanol/water 1/1 (v/v).

The hop acids standard was purchased from 'Versuchsstation Schweizerischer Brauereien' (Zurich, Switzerland) and contained 17.69% cohumulone, 41.49% adhumulone and humulone, 9.66% colupulone, and 8.46% adlupulone and lupulone. The standard (mixture of humulones and lupulones; 1.27 mg/mL) was dissolved in methanol prior to use.

2.3 CEC

Analyses were carried out using a Hewlett Packard HP^{3D} Capillary Electrophoresis System with diode array detector (Hewlett Packard, Waldbronn, Germany). Packed capillaries were also obtained from Hewlett Packard. Capillary dimensions were: 100 µm ID, 3 µm C18 particles, 335 mm total length and 250 mm effective length. The mobile phase (also used for conditioning) consisted of

10 mM Tris in 0.5% (v/v) formic acid in water/acetonitrile 40/60 (v/v). Injections were made hydrodynamically by applying 7 bar at the inlet of the capillary for 0.2 min. Both ends of the capillary were pressurized with 9 bar during the analyses to suppress bubble formation. The applied voltage was 30 kV and the temperature was set at 30°C. The electroosmotic flow (thiourea as marker) was 1.21 mL/s. DAD was used as detection and detection wavelengths were set at 280 nm and 340 nm.

2.4 HPLC

HPLC was performed on a HP1100 Series equipped with a variable wavelength detector (Hewlett Packard, Waldbronn, Germany). An Alltima C18 column, 4.6 mm × 250 mm, 5 µm particle size (Alltech, Lokeren, Belgium) was used. The mobile phase consisted of 1% formic acid in water (solvent A) and acetonitrile (solvent B). The flow rate was set at 1 mL/min. Gradient elution was applied with the following gradient: 0–2 min: 45% B isocratic, 2–20 min: 45–75% B, 20–25 min: 75–95% B, 25–30 min: 95% B isocratic, 30–35 min: 95–45% B. Injections of the hop extract (20 µL) were done automatically.

3 Results and discussion

The hop acids standard was used during method development. The humulones can be readily distinguished from the lupulones by their UV features (**Figure 2**). Separation between the critical pair of isomers humulone/adhumulone is satisfactory, while the lupulone/adlupulone isomers are even baseline separated. A disadvantage is the increased peak width of the β-acids in line with the prolonged analysis time. However, the β-acids are much less interesting than the α-acids, as the latter are precursors for the bitter-tasting iso-α-acids. The repeatability of the retention times was ex-

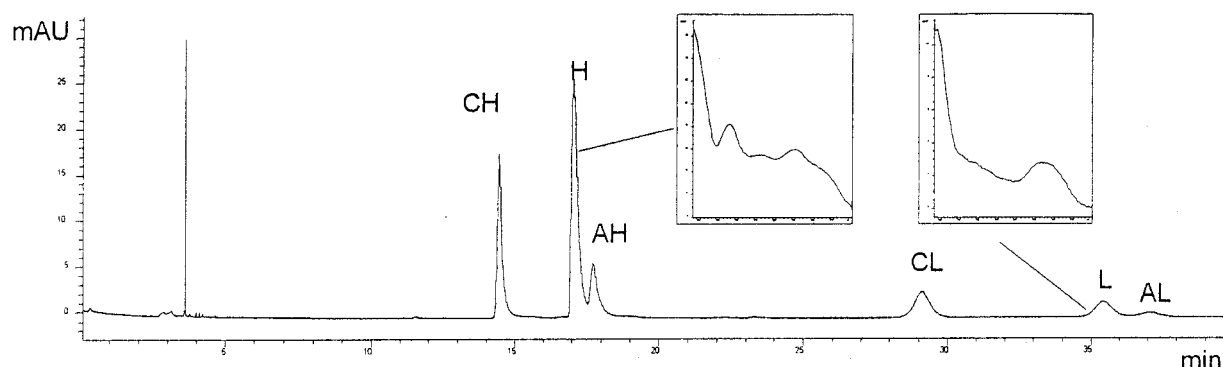


Figure 2. Chromatogram of the CEC analysis of a hop acids standard mixture with UV spectra of humulone and lupulone inserted. Detection at 340 nm. Other experimental conditions: see 'Materials and methods'. Peak identification: CH = cohumulone, H = humulone, AH = adhumulone, CL = colupulone, L = lupulone, AL = adlupulone.

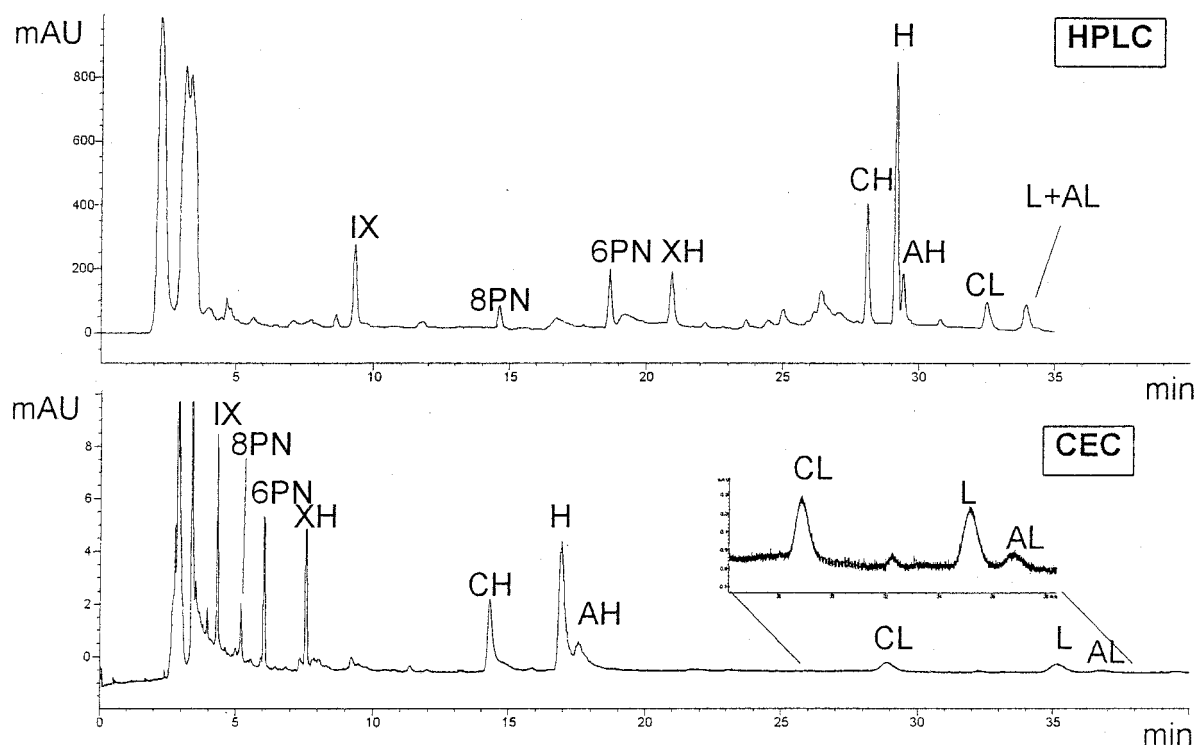


Figure 3. Comparison of HPLC and CEC analyses of a polyphenolic extract of the hop variety Hallertau Magnum. Detection at 280 nm. Other experimental conditions: see 'Materials and methods'. Peak identification: IX = isoxanthohumol, 8PN = 8-prenylnaringenin, 6PN = 6-prenylnaringenin, other codes: see Figure 2.

Table 1. Repeatability of retention times (5 injections) of a hop acids standard mixture. Experimental conditions and abbreviations: see Figure 2.

Injection no.	Retention time (min)					
	CH	H	AH	CL	L	AL
1	14.5	17.2	17.8	29.3	35.8	37.3
2	14.4	17.0	17.6	29.0	35.2	37.0
3	14.4	17.0	17.6	29.0	35.1	36.9
4	14.5	17.2	17.8	29.4	35.7	37.3
5	14.4	17.0	17.7	29.1	35.4	37.0
Average (min)	14.4	17.1	17.7	29.2	35.4	37.1
RSD (%)	0.38	0.64	0.56	0.62	0.86	0.50

cellent (**Table 1**), but RSD values for the areas were up to 30%, probably due to malfunctioning injection. Work is in progress to ameliorate the quantitation.

Next, we investigated real samples using the conditions optimized with the standard. Our hop extracts contain the

hop acids as well as prenylated flavonoids including the chalcone xanthohumol and the flavanones isoxanthohumol, 8-prenylnaringenin, and 6-prenylnaringenin (Figure 1). Clearly, all hop acids and prenylated hop flavonoids are well separated by CEC. Individual constituents were identified by spiking with authentic compounds and by the respective UV spectra. A most appropriate comparison between CEC and HPLC would, amongst others, call for the use of identical packing material. The HPLC analysis, shown in **Figure 3**, is given only to highlight that the elution order of all components is identical to that observed in CEC. In addition, it may be noted that, under the HPLC conditions used, lupulone and adlupulone are not resolved.

4 Conclusion

The CEC-method presented here enables to separate all hop acids (α - and β -acids) and the main prenylated flavonoids, present in polyphenolic hop extracts, in a single run. The procedure should be readily applicable to ana-

lyse commercial hop products for their content of both taste-active and bioactive constituents.

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