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Short communication

Sensitive electrochemical detection method for α-acids, β-acids and xanthohumol in hops (*Humulus lupulus* L.)

Javor Kac *, Tomaž Vovk

Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, SI-1000 Ljubljana, Slovenia

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Abstract

A new HPLC method with coulometric detection for the quantification of xanthohumol, α-acids and β-acids in hops was developed. The separation of compounds was accomplished with a C_{18} column and isocratic elution with methanol: 50 mM potassium phosphate: ortho-phosphoric acid = 80:20:0.25 (v/v/v). The method was validated and UV and electrochemical detectors (ECD) were compared. The HPLC method with ECD was precise, accurate and very sensitive for detection of xanthohumol and α- and β-acids. The detection limits of analytes were at least 8.8 to 24 times lower with ECD than those of the UV detector. The ECD method was successfully applied for quantification of studied compounds in hop pellets. The concentrations of all compounds obtained with ECD and UV were found to be equivalent. This is the first study demonstrating a very sensitive and validated method for the quantification of xanthohumol, α- or β-acids in hop samples with the use of the electrochemical detector.

Keywords: Hops; Xanthohumol; Humulones; Lupulones; Electrochemical detector; UV detector

1. Introduction

Hop (*Humulus lupulus* L.) is a dioecious perennial plant that grows wild in Europe and North America, and is also widely cultivated. The female inflorescences are used in the brewing industry to add bitterness and flavour to beer. The bitterness originates from α- and β-acids that are present in hop strobiles. Besides hop usefulness in beer industry hop components exhibit many pharmacological activities, the antioxidant action being one of the most promising. There are some reports of the antioxidant action of α- and β-acids [1], however at the moment xanthohumol (XN) is the most studied hop component with antioxidant action and potential therapeutic usefulness [2–6].

Quantification of xanthohumol, α- and β-acids in hop samples has so far been done by using high performance liquid chromatography (HPLC) with UV or MS detection [7–9], and high performance thin layer chromatography [10]. Non-aqueous capillary electrophoresis was also used in analysis of xanthohumol and β-acids [11]. In the beer industry, hop acids are analysed with HPLC and UV detection at 314 nm [12]. The following substances are then reported: cohumulone (coH), ad- and *n*-humulone (H), colupulone (coL), and ad- and *n*-lupulone (L) (Fig. 1).

Because of antioxidant properties of the studied compounds, electrochemical detector (ECD) with coulometric detection was considered as a potential sensitive method. So far the electrochemical detection has not been reported for α-acids, β-acids, or xanthohumol. ECD was used in determination of 19 phenolic acids in wort and beer [13]. The HPLC method with ECD was reported for the analysis of 27 phenolic compounds in beer, including derivatives of benzoic and cinnamic acids, flavones, and a few related glycosides [14]. In this work we used HPLC with ECD to develop a sensitive method for determination of α-acids, β-acids and xanthohumol.

2. Experimental

2.1. Chemicals and samples

Water for all applications was obtained from an Elga PURELAB classic purification system (High Wycombe, UK) and its resistivity was equal to or higher than 18.2 MΩ cm. All chemicals used were of analytical grade. Potassium dihydrogen phosphate and ortho-phosphoric acid were pur-

* Corresponding author. fax: +386 1 4258 031.
E-mail address: javor.kac@ffa.uni-lj.si (J. Kac).

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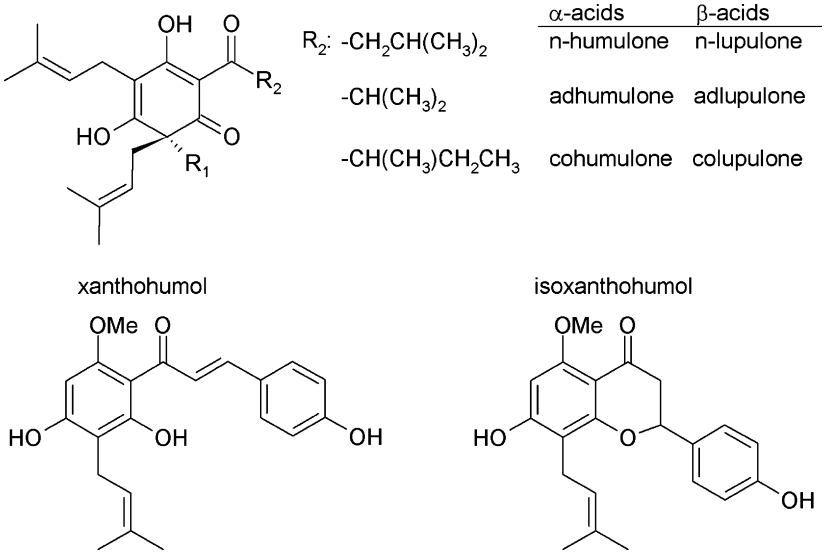

Fig. 1. Chemical structures of α-acids (R_1 = —OH), β-acids (R_1 = prenyl), xanthohumol (XN) and isoxanthohumol. Other abbreviations used in the text: coH (cohumulone), H (ad- and *n*-humulone), coL (colupulone), L (ad- and *n*-lupulone).

chased from Merck (Darmstadt, Germany). Methanol was Chromasolv® from Sigma–Aldrich (Buchs, Switzerland). Xanthohumol standard (>99% HPLC) was from N.I.C. (Homburg, Germany). Hop acids standard was a mixture of α-acids and β-acids of well-known composition, ICE-2 (14.45% cohumulone, 34.94% (*n*-humulone + adhumulone), 12.92% colupulone, 12.02% (*n*-lupulone + adlupulone)), obtained from Versuchsstation Schweizerische Brauereien (Zürich, Switzerland).

Type 90 hop pellets of cultivars Savinjski Golding (crop 2003), Magnum (crop 2003) and Aurora (crop 2003 and 2004) were obtained from Pivovarna Laško brewery (Laško, Slovenia). They were kept at +5 °C until analysed.

2.2. Chromatographic system

The HPLC system Agilent 1100 series (Waldbronn, Germany) consisted of the following units: isocratic pump, vacuum degasser, column thermostat, autosampler and UV detector. Additionally a CoulArray Model 5600 (Chelmsford, MA, USA) electrochemical detector equipped with an analytical cell Model 6210 was coupled to the system. The analytical cell consisted of four serial carbon porous electrodes and potentials of 0, −500, −500 and 700 mV (versus palladium reference electrode) were applied on the 1st–4th electrodes, respectively. The chromatographic separation was obtained with a C_{18} reversed-phase column (Phenomenex Gemini, 150 mm × 4.6 mm, 5 μm) that was connected to the Hypersil C_{18} precolumn. The isocratic mobile phase used in Method of EBC 7.7 [12] was modified to allow coulometric detection. The mobile phase consisted of methanol: 50 mM potassium phosphate: ortho-phosphoric acid = 80:20:0.25 (v/v/v). Prior to use, the mobile phase was filtered through a 0.45 μm filter under vacuum and purged with helium. The flow rate of the mobile phase was 1 mL/min, the column temperature was 30 °C and the volume of injected samples was 20 μL. The UV detection was performed at 314 (for α-acids and β-acids) and at 368 nm (for xanthohumol) while electrochemical responses of all analytes were measured on the fourth electrode at 700 mV. The HPLC system was controlled by an HP ChemStation for LC, Rev.A.06.03, except for the CoulArray electrochemical detector which was controlled by ESA CoulArray for Windows 1.04.

2.3. Standards

A stock solution of XN (33 mg/L) was prepared in methanol and was later stored protected from light at 4 °C. A stock solution for α- and β-acids (2300 mg ICE-2 standard per L) was prepared by dissolving the appropriate amount of ICE-2 standard in methanol. Standards were prepared daily by dilution of the stock solutions of XN and mixture of α- and β-acids in methanol. Diluted methanol solutions were further diluted by the mobile phase (1:6, v/v) to provide working standards in the concentration range 0.7–307, 28–1730, 68–4170, 25–1540 and 23–1440 μg/L for XN, coH, H, coL and L, respectively.

2.4. Sample preparation

Samples of hop pellets were ground, 500 mg aliquots suspended in methanol (50 mL) and extracted for 10 min in an ultrasonic bath. A sample of the supernatant (∼2 mL) was withdrawn and filtered over a 0.45 μm cellulose acetate filter (Sartorius, Goettingen, Germany) prior to analysis. The samples were further diluted with methanol and mobile phase (1:6, v/v) and analysed.

2.5. Hydrodynamic voltammogram

The standard solution containing 0.11 mg/L of XN and 3.0 mg/L of the mixture of α-acids and β-acids was injected into the chromatographic system. The first, second and third electrodes were set at 0, 100 and 200 mV, respectively, while the potential of the fourth electrode was increased from 300 to

800 mV in increments of 100 mV. The standards were analysed at equal electrode potentials three times. To obtain the response of the measured compound at a specific potential, the responses at and below the given potential were summed in each chromatographic analysis and used for hydrodynamic voltammogram construction. For easier comparison of hydrodynamic voltammograms for all compounds their responses were recalculated to equal concentration of 0.1 mg/L.

2.6. Method validation

Six to ten working standards were analysed on each day of the method validation or sample analysis and used for construction of calibration curves. Calibration curves were prepared by plotting the peak height against standard concentrations and evaluated by linear regression analysis. In order to calculate intraday precision, three replicates of standards at three different concentrations were analysed in a single day, while the interday precision was obtained by analysing fresh standards daily for three days. The accuracy was obtained similarly; quality control samples at three concentration levels were analysed, their concentrations calculated by back calculation from the calibration curves and compared to their theoretical concentration. The limit of detection (LOD) was defined as the lowest detectable concentration, taking into consideration a signal-to-noise ratio of 3. The limit of quantification (LOQ) was considered a concentration with a signal-to-noise ratio of 10. The signal-to-noise ratio was determined from the chromatograms obtained by analysing standards at low concentration of analytes [15].

3. Results and discussion

In the light of the importance of hop components in beer industry as well as their potential use in medicine new sensitive methods for their quantification are needed. The quantification of α- and β-acids and XN using very sensitive coulometric detection presents an alternative to the classical UV HPLC method [12] especially when samples with low content or small amounts of sample are under investigation. The literature data regarding the analytical methods for studied compounds are very poor and except for the expensive LC–MS method [9,16,17] no other methods with LOD bellow 10 μg/L are reported. As the studied compounds, particularly XN, tend to have some redox activity we chose coulometric detection and compare it with well-known and used UV detection method. ECD with coulometric detection provides sensitivity and specificity for substances which are either oxidised or reduced at the applied potential. Additional improvement of detector specificity is achieved, when the array of electrochemical electrodes is employed. Our HPLC system enables simultaneous detection by UV and ECD detectors. Therefore, using a CoulArray detector we expected to demonstrate its high sensitivity and superiority over the traditionally used UV detector.

3.1. Chromatographic conditions

The chromatographic separation conditions originally used for determination of α- and β-acids by the European Brewery Convention [12] involve mobile phase consisting of methanol: water: ortho-phosphoric acid (85:17:0.25, v/v/v) and a C_{18} stationary phase. We attempt to transfer these chromatographic conditions of the original method to our chromatographic system and to adapt them in order to assure appropriate conductivity of the mobile phase needed for coulometric detection. The addition of the maximal KH_2PO_4 concentration to the mobile phase (10 mM) where no precipitation of the salt occurred enabled us to obtain high sensitivity of the ECD. Moreover, the addition of the phosphate salt to the mobile phase preserves good separation of the analytes previously obtained in the original method. The resulting chromatograms of samples are shown in Fig. 2 and responses of UV and ECD detectors present well-resolved peaks of XN (xanthohumol), coH (cohumulone), H (*n*-humulone + adhumulone), coL (colupulone) and L (*n*-lupulone + adlupulone) with retention times of 5.9, 12.3, 15.5, 23.3 and 30.3 min, respectively. The analytes' retention times were stable with RSD less than 1.9% during all study. Moreover, the retention times of analytes in samples differ by less than 2.1% from the retention times of standards which confirms peak identity in samples.

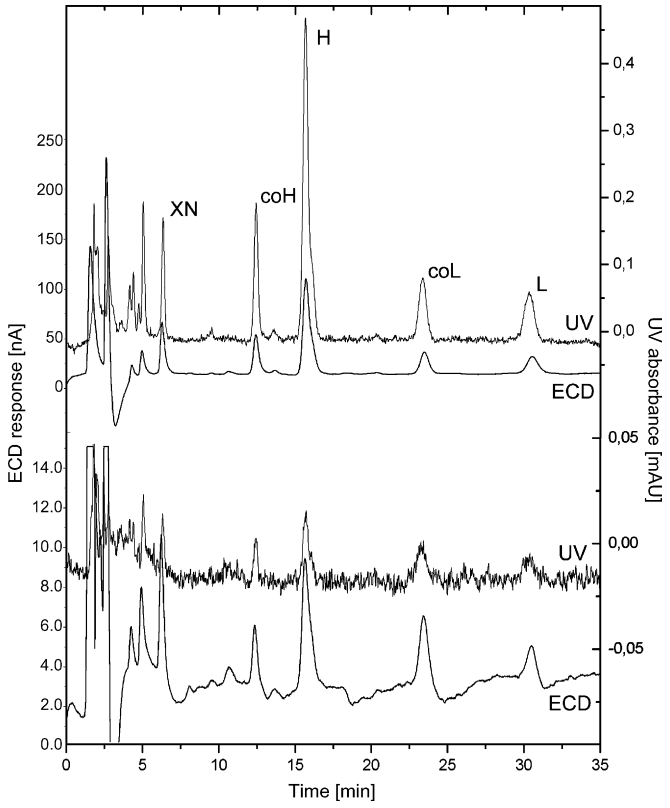

Fig. 2. Chromatograms obtained with typical sample concentration (top pair of curves) and diluted samples (bottom pair of curves), using ECD at +700 mV (top curve) and UV detection at 314 nm (bottom curve).

3.2. Electrochemical detection

The choice of the proper potential for analyte detection requires a compromise between sensitivity, enhanced by increasing voltage, and selectivity, reduced by increasing voltage. In

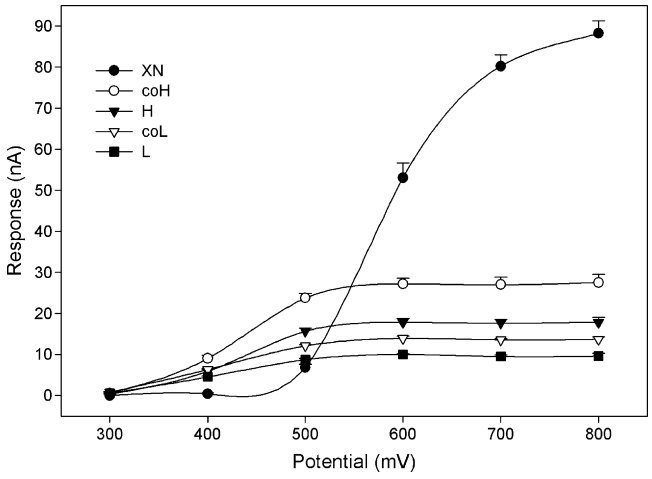

Fig. 3. Hydrodynamic voltammograms of XN, coH, H, coL and L. The analytes' responses obtained as described in Section 2.5 were recalculated to equal concentration 0.1 mg/L. The error bars represent the standard deviation of 3 replicates.

view of these considerations hydrodynamic voltammograms of studied compounds were constructed (Fig. 3) and this enabled us to select the optimal potential for maximal response of analytes. However, the potentials of the other three electrodes could also contribute to the obtaining of selective and sensitive detection. Particularly by concentrating the oxidation of the whole analyte amount in a single electrode the electrode response could be maximized. For the optimisation of responses xanthohumol was chosen and its response in analysis using different electrodes potentials was measured. We noted that while maintaining the first electrode potential at low positive values where XN is not yet oxidised, and the fourth electrode potential at values around the maximal XN response (700 mV), the response at the fourth electrode increased by about 20% if the second and third electrode potential was changed from 0 to a negative potential around −500 mV. Moreover, the above conditions also improved the XN peak symmetry (data not shown). Therefore, electrode potentials of 0, −500, −500 and +700 mV were chosen for electrodes 1 to 4, respectively.

3.3. Validation of HPLC method with ECD

The validation results for HPLC with ECD are summarized in Table 1. The intra-day precision expressed as RSD for all analytes at all studied concentrations were lower than 9% except for XN at lowest concentration where RSD was 16.7%. The results of the inter-day precision met the required criteria (RSD < 15%, except at the lowest concentration where RSD < 20%) for all compounds except L at the lowest concentration level. Moreover, acceptable accuracy and linearity were found for all analytes. The ECD demonstrated high sensitivity reaching the lowest LOD for XN, while for coH, H, coL and L the LOD were 4, 7, 8 and 17-fold higher, respectively. The same order as found in the determination of LOD could be seen in normalised hydrodynamic voltammograms (Fig. 3) where the highest response was obtained for XN and the lowest for L.

3.4. Comparison of ECD and UV detection

ECD was found to be about 20-fold more sensitive than UV at 314 nm for α- and β-acids (range: 16–24 fold) and 9-fold more sensitive than UV at 368 nm for XN. The sensitivity difference can be seen in Fig. 2 (bottom curves). The limits of detection for ECD were 0.1, 0.37, 0.64, 0.74, and 1.6 μg/L for XN, coH, H, coL, and L, respectively. The order of LOD was the same as that in normalized hydrodynamic voltammograms (Fig. 3). The LOD for XN was much lower when compared to LOD 13 μg/L obtained by Avula et al. using HPLC with UV detection [18]. The limits of detection for all compounds were comparable to those reported for LC–MS. For example, LC–MS was used for xanthohumol in the range from 10 to 800 μg/L [9], and for α-acids and β-acids with the LOD 10 and 5 μg/L, respectively [16]. Zhang et al. [17] reported on a LOD 0.04 μg/L for colupulone in the analysis of hop bitter acids by HPLC coupled with atmospheric pressure ionization tandem mass spectroscopy.

Tables 1 and 2, respectively, summarize the validation parameters for ECD and UV detectors. The intra-day precisions obtained with ECD and UV detectors were comparable for both modes and for all compounds studied at three levels except at lowest concentration where better precision was achieved with ECD. Moreover, XN could not be quantified with UV detection at the lowest level. The RSD for both detection modes was acceptable (RSD below 15% except at lowest concentration).

Inter-day precision was found to be satisfactory (RSD below 15% except at lowest concentration) for all compounds at three levels with both detection modes.

The accuracy of both methods was investigated by means of quality control samples at three levels. Mean biases were between −10% and +10% for both detection modes and all compounds at three levels.

A linear relationship was found between the heights and the injected concentration for all compounds, at the assayed range (see Tables 1 and 2).

Using 24 hop extracts, the results for each of the peaks obtained with two methods were compared. The extracts were analysed and the concentration of each compound was calculated using the calibration curve obtained in the same day. For each compound the concentrations found using ECD data were plotted against the concentrations obtained using UV data. Linear curves were then fitted to these data and for all analytes the slopes, intercepts and Pearson's correlation coefficients were in the range of 0.984–1.014, -5.77×10^{-4} to $+9.53 \times 10^{-3}$ and 0.9982–0.9998, respectively. The slopes are very near unity for all compounds with maximal bias of 1.6%. The intercept is close to zero and the Pearson's correlation coefficient is greater than $R = 0.998$ for all compounds. These results show that ECD and UV methods are equivalent with regards to the results they produce.

3.5. Sample analysis

Two batches of four samples of different hop cultivars or crops were extracted. The content of XN, coH, H, coL, and L was determined using coulometric and UV detection after trip-

Table 1
Validation results for HPLC with ECD detection at +700 mV

	Xanthohumol			Cohumulone			*n*- ad-humulone			Colupulone			*n*- ad-lupulone		
	Concentration (μg/L)	Response (nA)	RSD (%)	Concentration (μg/L)	Response (nA)	RSD (%)	Concentration (μg/L)	Response (nA)	RSD (%)	Concentration (μg/L)	Response (nA)	RSD (%)	Concentration (μg/L)	Response (nA)	RSD (%)
Intra-day precision ($n=3$)	0.72	0.77 ± 0.13	16.7	28.2	7.52 ± 0.12	1.66	68.1	16.1 ± 0.3	1.90	25.2	3.9 ± 0.4	8.99	23.4	2.3 ± 0.2	8.15
	23.1	23.1 ± 0.4	1.52	247	68.6 ± 1.1	1.57	596	90.1 ± 0.6	0.71	220	26.4 ± 0.2	0.58	205	20.1 ± 0.6	2.92
	115.3	120.3 ± 1.5	1.27	1726	474.7 ± 9.5	2.00	4173	599.3 ± 13.6	2.28	1543	185 ± 3	1.65	1436	148.0 ± 1.0	0.68
	Sample[a]	33.6 ± 0.6	1.64	Sample[a]	118.3 ± 1.5	1.29	Sample[a]	288.0 ± 4.0	1.39	Sample[a]	65.3 ± 0.9	1.37	Sample[a]	49.7 ± 0.5	1.01
Inter-day precision ($n=3$)	1.98	1.61 ± 0.11	6.56	28.2	8.2 ± 1.5	18.7	68.1	13.1 ± 2.6	19.5	25.2	3.6 ± 0.3	8.21	23.4	2.5 ± 0.6	25.6
	23.1	24.4 ± 1.2	4.78	247	63.4 ± 6.7	10.6	596	101.4 ± 10.1	9.96	220	29.2 ± 2.5	8.61	205	19.5 ± 1.6	8.25
	115.3	126.9 ± 6.3	5.00	1726	445 ± 63	14.1	4173	714 ± 99	13.9	1543	204 ± 18	8.60	1436	138 ± 12	8.51

Accuracy ($n=3$)	Concentration (μg/L)	Bias ± SD (%)	Concentration (μg/L)	Bias ± SD (%)	Concentration (μg/L)	Bias ± SD (%)	Concentration (μg/L)	Bias ± SD (%)	Concentration (μg/L)	Bias ± SD (%)
	1.83	+0.72 ± 4.77	61.5	+8.91 ± 0.42	149	+9.21 ± 1.83	55.0	−4.71 ± 5.82	51.2	+4.0 ± 12.3
	11.5	−0.36 ± 1.99	345	+1.15 ± 0.07	833	+1.60 ± 0.94	308	+0.48 ± 1.02	287	+1.30 ± 1.26
	61.5	+5.09 ± 0.84	1378	−0.80 ± 1.68	3333	−0.56 ± 2.22	1232	−1.66 ± 0.85	1147	−2.37 ± 1.89
Regression analysis[b]	$y = 1.0188 \pm 0.0089x - 0.00037 \pm 0.00018$		$y = 0.274 \pm 0.002x - 0.197 \pm 0.148$		$y = 0.183 \pm 0.001x - 0.443 \pm 0.414$		$y = 0.137 \pm 0.001x - 0.035 \pm 0.154$		$y = 0.0994 \pm 0.0008x - 0.256 \pm 0.210$	
	$R = 0.99969$		$R = 0.99985$		$R = 0.99987$		$R = 0.99979$		$R = 0.99988$	
Range	0.72–307 μg/L		28.2–1726 μg/L		68.1–4173 μg/L		25.2–1543 μg/L		23.4–1436 μg/L	
LOD	0.1 μg/L		0.37 μg/L		0.64 μg/L		0.74 μg/L		1.6 μg/L	
LOQ	0.32 μg/L		1.2 μg/L		2.1 μg/L		2.5 μg/L		5.3 μg/L	

[a] The sample data are for Aurora (crop 2004).
[b] The number of points in the calibration curves was 10 for xanthohumol and 6 for other substances. Each point was the mean of three experimental measures.

Table 2
Validation results for HPLC with UV detection

	xanthohumol			cohumulone			*n*- ad-humulone			colupulone			*n*- ad-lupulone		
	Conc. (μg/L)	Response (mAU)	RSD (%)	Conc. (μg/L)	Response (mAU)	RSD (%)	Conc. (μg/L)	Response (mAU)	RSD (%)	Conc. (μg/L)	Response (mAU)	RSD (%)	Conc. (μg/L)	Response (mAU)	RSD (%)
Intra-day precision ($n=3$)	0.72	Below LOQ	–	28.2	0.042 ± 0.003	6.47	68.1	0.060 ± 0.004	7.05	25.2	0.016 ± 0.002	10.4	23.4	0.013 ± 0.003	21.8
	23.1	0.244 ± 0.004	1.50	247	0.352 ± 0.003	0.80	596	0.548 ± 0.006	1.05	220	0.130 ± 0.005	3.85	205	0.088 ± 0.002	2.36
	115.3	1.267 ± 0.007	0.52	1726	2.398 ± 0.026	1.08	4173	3.726 ± 0.042	1.14	1543	0.883 ± 0.013	1.50	1436	0.599 ± 0.007	1.22
	Sample[a]	0.342 ± 0.010	2.80	Sample[a]	0.58 ± 0.01	2.08	Sample[a]	1.39 ± 0.02	1.26	Sample[a]	0.266 ± 0.003	1.15	Sample[a]	0.208 ± 0.001	0.40
Inter-day precision ($n=3$)	1.98	Below LOQ	–	28.2	0.044 ± 0.007	16.4	68.1	0.065 ± 0.014	22.0	25.2	0.017 ± 0.003	15.6	23.4	0.011 ± 0.002	19.8
	23.1	0.242 ± 0.007	2.94	247	0.32 ± 0.03	10.3	596	0.50 ± 0.05	10.0	220	0.122 ± 0.009	7.07	205	0.082 ± 0.005	6.65
	115.3	1.26 ± 0.05	4.03	1726	2.23 ± 0.30	13.6	4173	3.48 ± 0.45	12.9	1543	0.85 ± 0.06	7.45	1436	0.58 ± 0.04	6.93
Accuracy ($n=3$)	Conc. (μg/L)	Bias ± SD (%)		Conc. (μg/L)	Bias ± SD (%)		Conc. (μg/L)	Bias ± SD (%)		Conc. (μg/L)	Bias ± SD (%)		Conc. (μg/L)	Bias ± SD (%)	
	1.83	Below LOQ		61.5	+2.42 ± 3.35		149	+1.31 ± 1.83		55.0	−3.46 ± 1.53		51.2	+2.91 ± 5.86	
	11.53	+1.49 ± 2.17		345	+0.65 ± 2.38		833	+1.20 ± 3.00		308	+1.93 ± 3.31		287	+3.50 ± 2.11	
	61.48	+3.41 ± 1.34		1378	−2.39 ± 3.84		3333	−2.22 ± 3.36		1232	−0.04 ± 2.92		1147	−0.87 ± 3.65	
Regression analysis[b]	$y = 0.01118 \pm 0.00006x$ − 0.00538 ± 0.00424			$y = 0.00139 \pm 0.000005x$ + 0.000737 ± 0.00119			$y = 0.000896 \pm 0.000002x$ − 0.00394 ± 0.00152			$y = 0.000574 \pm 0.000002x$ + 0.000633 ± 0.000283			$y = 0.000418 \pm 0.000002x$ +0.000389 ± 0.000738		
	$R = 0.99991$			$R = 0.99998$			$R = 0.99999$			$R = 0.99996$			$R = 0.99993$		
Range	3.46–307 μg/L			28.2–1726 μg/L			68.1–4173 μg/L			25.2–1543 μg/L			117–1436 μg/L		
LOD	0.88 μg/L			7.7 μg/L			12 μg/L			18 μg/L			25 μg/L		
LOQ	4.1 μg/L			26 μg/L			40 μg/L			60 μg/L			84 μg/L		

The detection wavelength was 368 nm for xanthohumol and 314 nm for other compounds.

[a] The sample data are for Aurora (crop 2004).

[b] The number of points in the calibration curves was 8 for xanthohumol and 6 for other substances. Each point was the mean of three experimental measures.

Table 3
Content comparison for the studied compounds in four samples of hop pellets obtained from the UV and ECD results

		Savinjski Golding (2003)	Magnum (2003)	Aurora (2003)	Aurora (2004)
Xanthohumol	UV[a]	0.177 ± 0.022[b]	0.182 ± 0.011	0.288 ± 0.043	0.309 ± 0.027
	ECD	0.176 ± 0.022	0.184 ± 0.013	0.306 ± 0.036	0.312 ± 0.027
Cohumulone	UV	0.486 ± 0.010	1.405 ± 0.038	1.142 ± 0.045	1.284 ± 0.033
	ECD	0.484 ± 0.013	1.383 ± 0.044	1.132 ± 0.060	1.268 ± 0.048
n- ad-humulone	UV	1.540 ± 0.045	4.014 ± 0.116	4.488 ± 0.203	4.731 ± 0.131
	ECD	1.511 ± 0.057	3.981 ± 0.142	4.466 ± 0.240	4.717 ± 0.167
Colupulone	UV	1.000 ± 0.027	1.830 ± 0.043	1.594 ± 0.064	1.420 ± 0.028
	ECD	0.989 ± 0.039	1.814 ± 0.058	1.581 ± 0.076	1.412 ± 0.047
n- ad-lupulone	UV	0.966 ± 0.032	2.651 ± 0.067	1.820 ± 0.071	1.492 ± 0.033
	ECD	0.954 ± 0.019	2.606 ± 0.046	1.827 ± 0.063	1.501 ± 0.030

Crop year is given in brackets.
[a] 368 nm was used for xanthohumol and 314 nm for other compounds.
[b] Mean ± SD ($n = 6$).

licate injection of each batch. The results for four samples are summarized in Table 3. Both methods gave similar results for all studied compounds. XN content ranged from 0.18% in Savinjski Golding cultivar (crop 2003) to 0.31% in Aurora cultivar (crop 2004). coH content was from 0.48% to 1.38% in Magnum (2003). The content of H ranged from 1.51% to 4.72% in Aurora (2004). coL was present in the range from 0.99% to 1.81% in Magnum (2003). The lowest amount of L was 0.95% found in Savinjski Golding (2003) and the highest was 2.61% found in Magnum (2003). The content of studied compounds was lower than expected in fresh hops, possibly because of the decomposition due to age.

4. Conclusion

This study was performed in order to compare the classically used UV HPLC method with our new proposed HPLC method with coulometric detection for quantification of XN, and α- and β-acids in hops. The modified chromatographic conditions enabled us to achieve good separation of analytes and appropriate conditions for coluometric detection. The proposed HPLC method with ECD is precise, accurate and most important very sensitive for detection of xanthohumol and α- and β-acids. The detection limits of analytes were at least 8.8 to 24 times lower with ECD than those of the UV detector and were comparable with the most sensitive methods reported in the literature. The validated method was successfully applied to quantify analytes in hop pellets and their concentrations obtained with coulometric and UV detection were equivalent.

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