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Quantitative analysis of xanthohumol and related prenylflavonoids in hops and beer by liquid chromatography–tandem mass spectrometry

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Abstract

A method for quantitation of six prenylflavonoids (xanthohumol, isoxanthohumol, desmethylxanthohumol, 6- and 8-prenylnaringenins and 6-geranylnaringenin) in hops and beer by HPLC–tandem mass spectrometry has been developed. The method allows direct analysis of beer and crude methanolic extracts of hops. After HPLC separation, prenylflavonoids were detected by positive ion multiple-reaction monitoring using a triple-quadrupole mass spectrometer equipped with a heated nebulizer–atmospheric pressure chemical ionization interface. The accuracy and precision were evaluated by replicate analyses of (spiked) samples. Thirteen commercial beers were analysed with the method. Isoxanthohumol, formed by isomerization of xanthohumol during the brewing process, was the most abundant flavonoid in hopped beers, ranging from 0.04 to 3.44 mg/l. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Hops, the female inflorescences of the hop plant (*Humulus lupulus* L.), are used in the brewing industry to add bitterness and aroma to beer. Most research on beer flavour has therefore been focused on bitter acids and essential oils. It has been suggested that the hop flavonoids moderate beer bitterness and increase stability, but the brewing value of these polyphenolics is not well understood [1]. Xanthohumol and other prenylated flavonoids from hops [2] have recently received attention because of their potential anti-cancer properties [3–5].

Little is known about the dietary intake (estimates vary from 50 mg to about 1 g per person per day [6]) and pharmacokinetics of flavonoids in general [7]. Food products such as vegetables and fruits contain mainly glycosides of common flavonols, flavones and flavanones [8]. Prenylated flavonoids are found in a limited number of plant families, of which the Moraceae-Cannabinaceae (with *Humulus lupulus*), Leguminosae and Asteraceae host roughly 80% of the ca. 1100 known prenylflavonoids [9]. Although a number of food plants belong to prenylflavonoid-bearing families, none of these can be identified as a rich nutritional source of these phenolics in the diets of western countries. While the presence of pre-

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nylflavonoids in beer has not been reported in the literature previously, beer may well represent the most important source of dietary prenylflavonoids.

Xanthohumol has been quantified in hops by high-performance liquid chromatography (HPLC) with UV detection [10], but this technique offers insufficient sensitivity and selectivity for quantitative analysis of the minor prenylflavonoids in beer. As a detection technique, tandem mass spectrometry (MS–MS) can provide improved sensitivity, and its greater selectivity makes it especially useful for analysis of minor components in complex matrices.

Liquid chromatography (LC) coupled with (tandem) mass spectrometry has been successfully applied to the quantitative analysis of soya isoflavones in plasma [11] and in baby foods and soya flour [12]. We now wish to report on the development of a method for quantitation of xanthohumol and five other prenylflavonoids in hops and beer (Fig. 1) by HPLC–MS–MS. To demonstrate the suitability of the method, a selection of beers and two hop-containing herb teas have also been analysed.

2. Experimental

2.1. Flavonoid standards and reagents

Crude xanthohumol was isolated from hops as before [2], which contained small amounts of desmethylxanthohumol and 2′,4′,6′,4-tetrahydroxy-3′-geranylchalcone in addition to xanthohumol. Isoxanthohumol was prepared by isomerization of xanthohumol as follows. Crude xanthohumol was dissolved in 1% aqueous sodium hydroxide at 0°C (ca. 15 mg/ml). After 10 min, formic acid (50 μl per ml of 1% sodium hydroxide) was added to stop the reaction. Acetonitrile was added to the reaction mixture to dissolve the precipitate. Preparative HPLC of the reaction mixture on RP-18 (10 μm Econosil 250×22 mm; Alltech, Deerfield, IL, USA), using a gradient from 40% to 80% acetonitrile in 1% aqueous formic acid over 30 min at 11.2 ml/min, yielded isoxanthohumol, 6-prenylnaringenin and 6-geranylnaringenin along with unreacted xanthohumol and desmethylxanthohumol. The products were de-

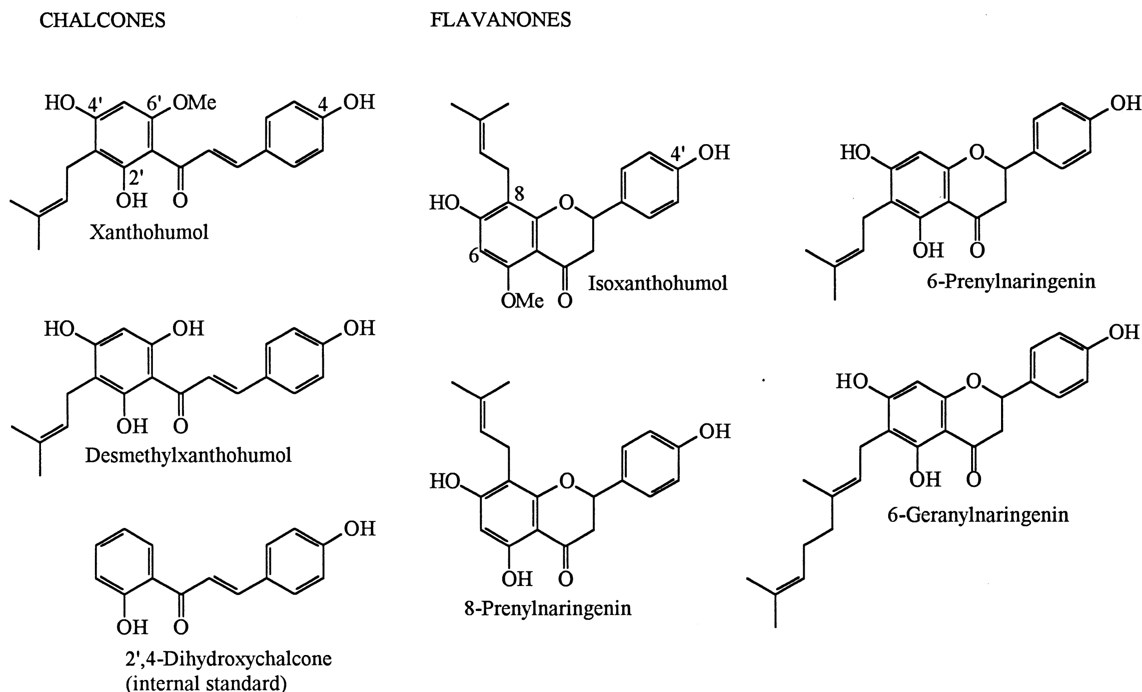

Fig. 1. Structures of chalcones and flavanones identified and quantified in this study.

tected at 254 nm, collected manually and recovered from the solvent by rota-evaporation and lyophilization. The identity and purity of the compounds were checked by analytical HPLC comparison with authentic flavonoids available in this laboratory [2]. The identity of 6-geranylnaringenin was established by MS and two-dimensional nuclear magnetic resonance (NMR) and UV spectroscopy. The remaining compound, 8-prenylnaringenin, was prepared by prenylation of naringenin [13]. Naringenin was obtained by hydrolysis of naringin (=naringenin-7-*O*-rhamnoglucoside, Sigma, St. Louis, MO, USA) in 2 *M* hydrochloric acid–methanol (1:1) under reflux conditions for 2 h.

HPLC-grade acetonitrile, methanol and dimethyl sulfoxide were obtained from Fisher (Fair Lawn, NJ, USA). Formic acid was from Fluka (Buchs, Switzerland), hydrochloric acid from EM Science (Cherry Hill, NJ, USA), ethyl ether from Mallinckrodt (Paris, KY, USA), boron trifluoride etherate, 2-methyl-3-buten-2-ol and 1,4-dioxane were from Acros (Pittsburgh, PA, USA). The internal standard, 2′,4-dihydroxychalcone, was purchased from Indofine (Somerville, NJ, USA).

2.2. Sample preparation

Liquid samples for LC–MS analysis were prepared by adding 50 μl of internal standard solution (1.0 mg 2′,4-dihydroxychalcone per ml of dimethyl sulfoxide) to 100 ml of liquid. Beers were purchased in local supermarkets. Beer samples were degassed by gentle swirling in a beaker followed by sonication for 2 min. After initial analysis, a few beers were diluted with ethanol–water (5:95, v/v) prior to addition of the internal standard in order to bring the prenylflavonoid concentrations into the mid-range of the calibration curves.

Two herb teas, both containing hops among their ingredients, were purchased in local stores. The quantitative composition of the teas was not mentioned on the labels. Teas were prepared by steeping one bag in 200 ml of boiling water for 5 min. After removal of the tea bag, the tea was allowed to cool to room temperature and spiked with the internal standard.

Hops (5 g, moisture content ca. 8%) were ground with a Wiley mill to pass through a No. 20 sieve. An aliquot (400 mg) was extracted with methanol (100 ml) by sonication for 10 min. A portion of the extract (ca. 4 ml) was filtered through a 0.2-μm nylon membrane filter (Nylaflo; Gelman, Ann Arbor, MI, USA). An aliquot of the filtrate (2.50 ml) was spiked with 50 μl of the internal standard and diluted to 100 ml with methanol–formic acid (99:1) before LC–MS analysis.

The precision was estimated by replicate analyses of the same sample preparations (within-sample precision) and analyses of replicate sample preparations of the same material (between-sample precision). For this purpose, ground hops were extracted five times and further processed as described above. One hop extract was selected for determination of the within-sample precision. Likewise, a homogeneous beer analyte was prepared by mixing the contents of two bottles and dividing the volume in five equal portions.

The accuracy was tested as follows. Since hop extracts were made up with methanol–formic acid (99:1, v/v), 100-ml aliquots of the latter solvent were spiked with known amounts of flavonoids. To test the accuracy of measured flavonoid levels in beer, known amounts of flavonoids were added to 100-ml aliquots of a ‘‘zero-flavonoid’’ beer (see Section 3.2).

2.3. HPLC

The HPLC system consisted of two Waters 6000A pumps (Milford, MA, USA) and an Altex Model 210 injector equipped with a 20-μl sample loop (Beckman, Berkeley, CA, USA). Separations were achieved on a Phenomenex (Torrance, CA) RP C_{18} column (250×4 mm, 5 μm) with a linear solvent gradient, starting on injection, from 40% to 100% B (acetonitrile) in A (1% aqueous formic acid) over 15 min, followed by 100% B for 5 min. The flow-rate was 0.8 ml/min. The column outlet was connected to a four-way valve (Upchurch, Oak Harbor, WA, USA). During the first 7 min of a chromatographic run, the column effluent was diverted to waste, while another solvent pump (Waters Model 6000A) connected to the four-way valve delivered solvent C (acetonitrile–1% aqueous formic acid, 1:1, v/v, 0.8 ml/min) to the heated nebulizer interface to protect it from overheating. After 7 min, the valve was

switched manually to introduce the column effluent directly into the interface.

2.4. Mass spectrometry

The PE Sciex API III Plus triple-quadrupole mass spectrometer (Perkin Elmer–Sciex Instruments, Thornhill, Ontario, Canada) was operated using the atmospheric pressure chemical ionization (APCI) source in the positive ion mode. Samples were introduced by HPLC via a heated nebulizer interface which was set to 500°C. Ionization of the analyte vapour mixture was initiated by a corona discharge needle at ca. 8 kV and a discharge current of ca. 3 μA. The orifice plate voltage was +55 V. MS–MS experiments were carried out with argon–nitrogen (9:1, Airco, Vancouver, WA, USA) as target gas at a thickness of ca. $1.8 \cdot 10^{14}$ atoms per cm^2. The collision energy was 30 V.

Quantitative MS–MS data were obtained by multiple reaction monitoring (MRM) of six flavonoids in three periods using the RAD version 2.5 software as follows. Period one (0–10.2 min): isoxanthohumol, m/z 355→179, and 2′,4-dihydroxychalcone, m/z 241→121; period two (10.2–13.65 min): desmethylxanthohumol, 6-prenylnaringenin and 8-prenylnaringenin, all m/z 341→165, and 2′,4-dihydroxychalcone, m/z 241→121; period three (13.65–20 min): xanthohumol, m/z 355→179, and 6-geranylnaringenin, m/z 409→165. The dwell time for each pair of parent–daughter ions was 200 ms, allowing 1.67 scans per s. Peak areas of the above prenylflavonoids were determined with the McQuan version 1.4 software, which was also used for further processing of the data.

Prenylflavonoid concentrations were calculated from calibration curves using 2′,4-dihydroxychalcone as the internal standard. Calibration standards were prepared by spiking methanol–formic acid (99:1, v/v) with a mixture of prenylflavonoids and the internal standard to give the following concentration levels: (i) 10, 50, 100, 400 and 800 μg/l for xanthohumol and isoxanthohumol; (ii) 2, 10, 20, 80 and 160 μg/l for desmethylxanthohumol, 6- and 8-prenylnaringenin; (iii) 1, 5, 10, 40 and 80 μg/l for 6-geranylnaringenin. These levels cover the typical concentrations of these compounds in hops and in beer. Desmethylxanthohumol was also made up separately to monitor its stability in methanol–formic acid (99:1, v/v). Standard solutions were stored at 4°C.

3. Results and discussion

3.1. Method development

A method for quantitation of xanthohumol and five other prenylflavonoids in hops and beer by liquid chromatography–tandem mass spectrometry has been developed. It allows very selective detection of these phenolics in crude hop extract and beer without sample clean up. In a previous study on tandem mass spectrometry of prenylflavonoids from hops [2], fragmentation of the $[MH]^+$ ions by collision-induced dissociation (CID) produced three daughter ions resulting from loss of the isoprenyl substituent, retro Diels-Alder (RDA) fission of the γ-pyranone ring, or both. In the present study, the collision energy was increased from 11 to 30 V leaving the product of both reactions, the $[A_1H-C_4H_8]^+$ ion, as the only major fragment (Fig. 2). This daughter ion was chosen for detection of the six prenylfavonoids (Table 1) in the MRM scan mode (Figs. 2–4). MRM is a tandem mass spectrometric detection technique in which an ion of a specific m/z value selected by the first mass analyzer is subjected to collision, and any fragments with an m/z ratio selected by the second mass analyzer are counted. This double-filtering process yields very selective data, while high sensitivity is gained by effective removal of unwanted ions (i.e., background). The MRM mode allows rapid sequential scanning of multiple ion pairs in a single LC run. Thus MRM permits co-elution of compounds with different masses or daughter fragments but cannot distinguish between 6-prenylnaringenin, 8-prenylnaringenin and desmethylxanthohumol (cf. Table 1). Therefore chromatographic separation was needed for separate detection of these isomeric prenylflavonoids (Figs. 3 and 4). This limited further shortening of the chromatographic run time to avoid losing too much resolving power. Another limitation against running a steeper gradient was contamination of the ion source by carbohydrates in beer. These hydrophilic macromolecules, which eluted in the first 5–7 min of

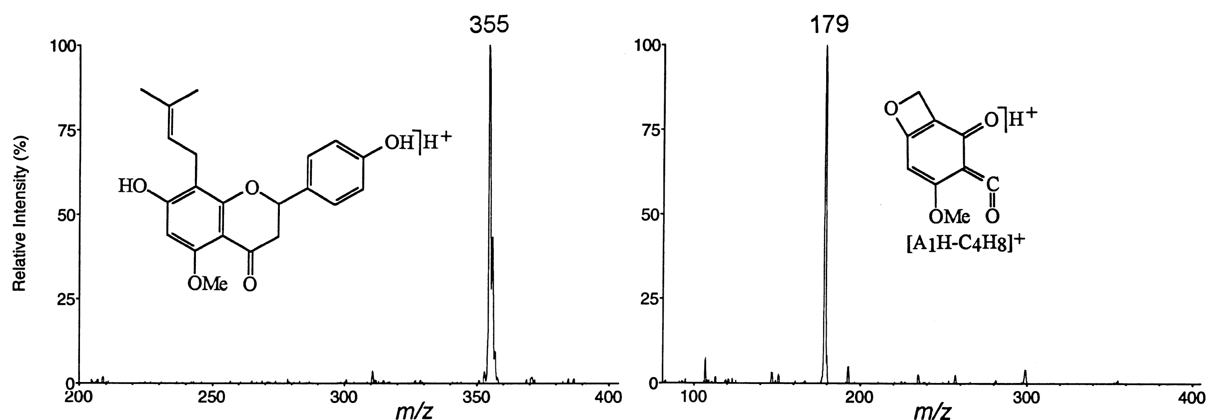

Fig. 2. Mass spectra of isoxanthohumol obtained using a heated nebulizer–atmospheric pressure chemical ionization interface. Single-quadrupole mass spectrum showing the quasi-molecular ion (left) and daughter-ion mass spectrum of isoxanthohumol obtained by collision-induced dissociation of the $[MH]^+$ ion with m/z 355.

a chromatographic run, were diverted to waste before the column eluate was directed to the mass spectrometer, thereby extending the lifetime of the quartz tube of the heated nebulizer.

Preliminary stability experiments revealed that xanthohumol and its desmethyl analogue are not stable in aqueous solutions. Xanthohumol and the isoprenylated flavanones can be kept in methanol for several weeks without noticeable loss, but desmethylxanthohumol and 6-geranylnaringenin are not stable in this solvent either. Although isomerization of chalcone type flavonoids into their isomeric flavanones accounts for most of the loss of xanthohumol, this was not the case with desmethylxanthohumol whose degradation was not in balance with formation of 6- and 8-prenylnaringenin. It was further observed that addition of 1% formic acid to aqueous or methanolic solutions slowed down the isomerization of xanthohumol, especially in aqueous solutions, and also increased the stability of desmethylxanthohumol and 6-geranylnaringenin in methanol considerably. Solutions containing desmethylxanthohumol still had to be made up fresh weekly, while standard solutions of the other pre-

Table 1
HPLC–MS–MS characteristics of six prenylflavonoids

Compound	HPLC Retention time (min)	Tandem MS		Standard curve[a]		
		Parent ion m/z	Daughter ion m/z	Regression equation[b]	No. of points	Correlation coefficient
Xanthohumol	14.18	355	179	$y=0.00820x$	12	0.99954
Isoxanthohumol	9.13	355	179	$y=0.00620x$	12	0.99979
8-Prenylnaringenin	11.63	341	165	$y=0.00399x$	15	0.99980
Desmethylxanthohumol[c]	12.45	341	165	$y=6.22\cdot10^{-6}x^2+0.001596x$	10	
6-Prenylnaringenin	13.27	341	165	$y=0.00348x$	15	0.99964
6-Geranylnaringenin	16.82	409	165	$y=0.00184x$	12	0.99842
4,2′-Dihydroxychalcone[d] (internal standard)	12.77	241	121			

[a] Regression statistics as reported by MacQuan quantification program on a typical day.
[b] y=Ratio of area of subject peak to area of internal standard peak, x=concentration of subject compound in micrograms per liter.
[c] Regression equation as calculated by SigmaPlot.
[d] Out of five 2′-hydroxychalcones, 4,2′-dihydroxychalcone was chosen as the internal standard because it produces an abundant RDA fragment (m/z 121) and elutes between isoxanthohumol and xanthohumol.

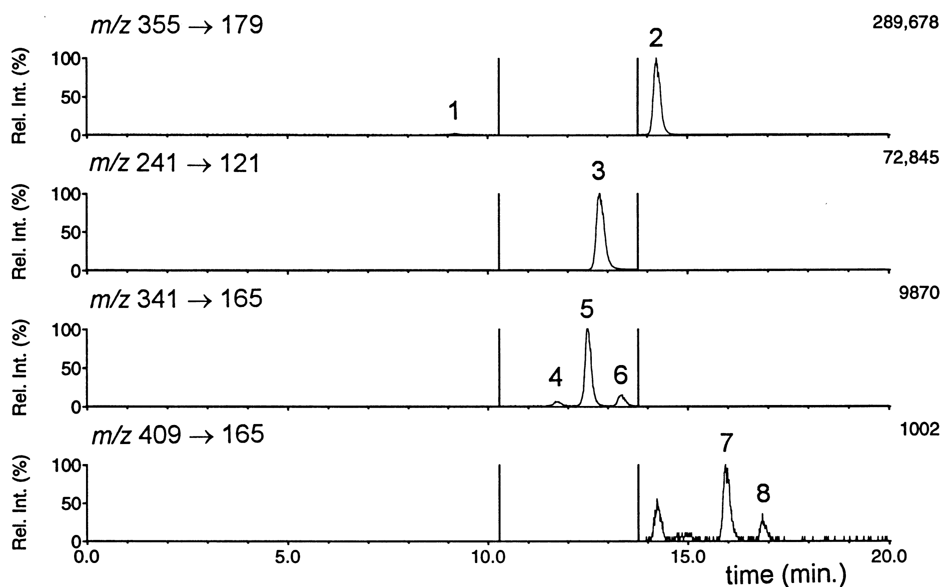

Fig. 3. HPLC–MS–MS analysis of a methanolic extract of hops. Peaks: 1=Isoxanthohumol, 2=xanthohumol, 3=2′,4-dihydroxychalcone (internal standard), 4=8-prenylnaringenin, 5=desmethylxanthohumol, 6=6-prenylnaringenin, 7=3′-geranylchalconaringenin, 8=6-geranylnaringenin. Prenylflavonoids were detected in a single HPLC run by multiple-reaction ion monitoring; vertical lines in the panels indicate start of a new scanning period. For further details, see Section 2.

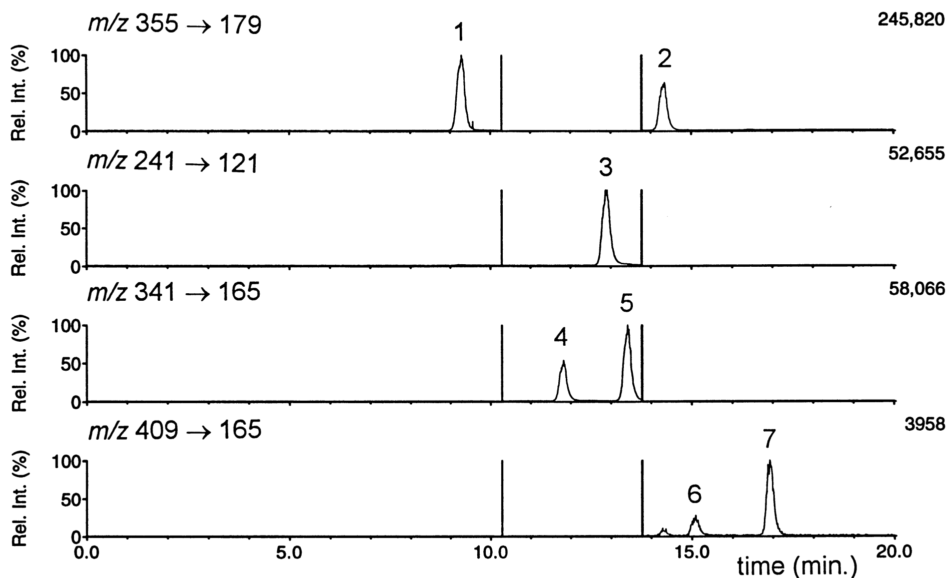

Fig. 4. HPLC–MS–MS analysis of a commercial beer. Peaks: 1=Isoxanthohumol, 2= xanthohumol, 3=2′,4-dihydroxychalcone (internal standard), 4=8-prenylnaringenin, 5=6-prenylnaringenin, 6=probably 8-geranylnaringenin, 7=6-geranylnaringenin. For further details, see Fig. 3.

nylflavonoids in methanol–formic acid (99:1, v/v) remained usable for up to four months.

The linear range of the detector was 0.01 to 1.0 mg/l for xanthohumol and isoxanthohumol, suitable for direct analysis of typical American hopped beers. Linearity was also observed with most minor prenylflavonoids at the lower levels normally found in hops and beer. The calibration data from three randomly selected days were studied in detail. The Student's t-test was used to determine if the calculated intercepts differed significantly from zero. Those for desmethylxanthohumol differed in every case (one-sided $p<0.05$) and those for the other compounds differed significantly in only one of 15 cases. A lack-of-fit F-test [14] was used to see how well the data fit a straight line. No significant lack-of-fit ($p>0.05$) was seen in 12 of 18 cases; for the other cases, $0.05 \geq p > 0.01$, including all three of the desmethylxanthohumol regression lines. For the above reasons, and because a zero response to blanks was observed and to avoid negative or exaggerated estimates at the low end of the concentration ranges, the results of MacQuan's ''linear through zero'' calculations were used for the estimated concentrations of xanthohumol, isoxanthohumol, 8- and 6-prenylnaringenin and 6-geranylnaringenin. The regression equations and correlation coefficients determined by MacQuan for a typical day are shown in Table 1.

The straight line produced by MacQuan for desmethylxanthohumol often visibly did not fit the data well: a scatter plot indicated a parabolic curve. A much better fit was achieved using a second-order polynomial equation (forced through zero) calculated and plotted by SigmaPlot 3.0. Estimated concentrations in the samples were then determined from the fitted data points calculated by SigmaPlot.

Inspection of the data demonstrated the need for using an internal standard in quantitative LC-MRM and for running standards each day. The peak area of the internal standard varied each day and between days: for example, from 1 099 122 to 1 325 761 ion counts on one day (C.V.=6.7%) and from 536 574 to 788 580 one day the following week (C.V. 10.8%). However, the peak area ratios varied much less: their C.V.s, averaged over all compounds and all concentrations, were 4.1% the first day and 3.6% the second day. The slopes of the calibration curves differed significantly ($p<0.01$, two-tailed Student t-test, all six flavonoids) between the two days, but the mean concentrations measured in five control samples were virtually identical.

3.2. Accuracy

The accuracy of the method was assessed by quantitation of known amounts of prenylflavonoids added to methanol–formic acid (99:1, v/v) and ''zero-flavonoid'' beer (beer No. 4, Table 4). These media were chosen to mimic hop extracts and beer, respectively. Comparison of the acidified methanol (=hop extract solvent) and beer data (Fig. 5) shows that the accuracy is better when prenylflavonoids are dissolved in acidified methanol at all concentration levels. With acidified methanol, the accuracy was within 10% of the expected concentrations except for 6-geranylnaringenin at the lowest concentration level. Thus, the method is suitable for quantitation of xanthohumol, desmethylxanthohumol, isoxanthohumol and both prenylnaringenins in methanolic extracts of hops.

The method also allows accurate analysis of the predominant prenylflavanones in beer, but the prenylchalcones and the apolar flavanone, 6-geranylnaringenin, yielded negative deviations greater than 10% from the expected concentrations. These findings could be explained by complexation of the more apolar flavonoids with macromolecules in beer. Beer contains about 4% carbohydrates and about 0.3% protein [15]. Complexation is a reversible process in which the equilibrium constant or % unbound flavonoid is dependent on the interactive forces between the macromolecule and the flavonoid and also on the water-solubility of the flavonoid. Only the unbound fraction is properly retained on the column, while the initial fraction of flavonoid adsorbed to macromolecules fails to participate in liquid–liquid distribution on the column and hence is eluted during the early minutes of a chromatographic run, escaping detection. Although there are ways to improve the accuracy (e.g., by depletion of the bound flavonoid pool by liquid–liquid extraction), the results indicate that this method is satisfactory for the analysis of the major prenylflavanones in beer without sample pretreatment. The method, however, underestimates the concentration of xanthohumol and desmethylxan-

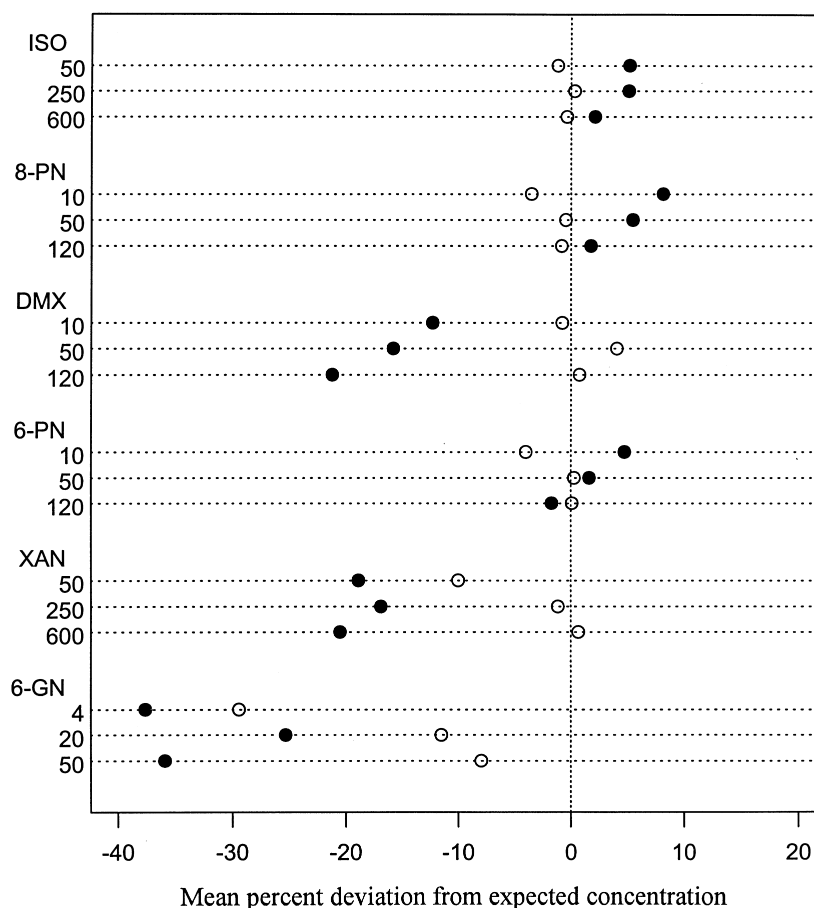

Fig. 5. Accuracy of HPLC–MS–MS analysis of prenylflavonoids in acidified methanol (○) and beer (●) shown as mean percent deviation from expected concentration, M%D=100·(measured conc.−expected conc.)/expected conc. Vertical axis indicates concentration levels in μg/l.

thohumol in beer. Isomerization in the brew kettle converts most xanthohumol into isoxanthohumol, and generally all desmethylxanthohumol into its isomeric prenylflavanones, 6- and 8-prenylnaringenin. This is why isoxanthohumol and the prenylnaringenins are more abundant in beer than the prenylchalcones, which predominate in hops (cf. Figs. 3 and 4).

3.3. Precision

One hop extract and one beer sample were analysed five times on a particular day in order to make an assessment of the within-day precision. As seen in Table 2, the precision ranged from 3.9 to 11.4%. 6-Geranylnaringenin, the least precise case, is only a trace component in hops. It is formed by isomerization (cyclization) of the more abundant hop flavonoid, 2′,4′,6′,4-tetrahydroxy-3′-geranylchalcone [2].

To study the variability of results from analyses on different days, one hop extract and one beer sample were analysed multiple times per day on several days over a one month period. The results were subjected to a one-way analysis of variance (ANOVA) (Table 3). In the methanolic hop extract, only isoxanthohumol showed significantly different results between days ($p=0.0035$). Inspection of the results in a scatter plot showed a steady increase in concentration, assumed to be caused by a slow isomerization of the much larger amount of xanthohumol. In

Table 2
Within-day precision of analysis of prenylflavonoids in hops and beer[a]

	Xanthohumol	Isoxanthohumol	8-Prenylnaringenin	Desmethylxanthohumol	6-Prenylnaringenin	6-Geranylnaringenin
Hop extract						
Mean (μg/l)	479.8	8.0	2.1	117.5	6.8	1.8
C.V.	3.9	7.2	6.4	5.6	8.5	11.4
Beer sample						
Mean (μg/l)	70.8	641.0	27.5	0.0	72.5	16.0
C.V.[b]	3.8	6.3	7.9		3.8	5.6

[a] One hop extract and one beer sample were each analyzed five times by HPLC–MS–MS on the same day.
[b] Coefficient of variation (C.V.)=100×standard deviation/mean concentration.

the beer sample, however, significant differences were found in the concentrations of many compounds when data from three or more widely spaced days were analysed in the ANOVA. This could be due to chemical, solubility, or complex-binding changes in the aqueous environment. Generally, however, analyses run on consecutive days or within a week yielded results that were not significantly different ($p>0.05$). Because of this, only two days of data were used in the between-day comparison of the selected beer sample shown in Table 3.

In another experiment, five hop and/or five beer samples were run on a single day. This was repeated three times within three weeks for both sets. The results were examined using scatter plots and two-way analysis of variance without replication. In most

Table 3
Precision of the HPLC–MS–MS analysis of prenylflavonoids

Sample	Df[a]	C.V.[b]					
		Xanthohumol	Isoxanthohumol	8-Prenylnaringenin	Desmethylxanthohumol	6-Prenylnaringenin	6-Geranylnaringenin
One hop extract							
Between day	4	4.7	15.8**	12.6	6.5	5.6	13.0
Within day	10	4.1	5.5	7.4	5.0	5.3	11.4
One beer sample							
Between day	1	6.9	2.6	6.0	–	2.1	8.0
Within day	4	4.7	3.6	5.9	–	4.7	6.2
All hop extracts							
Between day	2	4.6	19.6**	6.8	6.3**	1.4	11.4
Between sample	4	3.5	6.4	6.1	3.8*	2.0	12.5
Residual	8	2.6	4.6	7.1	1.5	4.6	8.4
All beer samples							
Between day	2	1.5	12.8*	8.2	–	3.5	4.8
Between sample	4	7.4	10.8*	8.8	–	8.9**	12.1
Residual	8	7.2	5.2	5.7	–	2.0	9.3

[a] Degrees of freedom in the analysis of variance.
[b] Coefficient of variation (C.V.), or relative root mean square, is the square root of the appropriate mean square from the analysis of variance, divided by the mean and multiplied by 100.
* Significant difference between groups, $p<0.05$.
** Significant difference between groups, $p<0.01$.

cases, no significant differences were observed between days or between samples. In the hop extracts, isoxanthohumol showed a steady increase over time, again probably a result of slow isomerization of the much more abundant xanthohumol. Of the beer samples, one was consistently different in its isoxanthohumol and 6-prenylnaringenin concentration; this cannot be readily explained in view of the manner of sample preparation. Also, a steady increase in the concentration of isoxanthohumol was seen which cannot be explained by isomerization because the smaller pool of xanthohumol remains constant. Some of the high C.V.s in Table 3 are a result of very small mean concentrations of, for example, 6-geranylnaringenin in hops.

3.4. Beer and herb tea analysis

Thirteen beers and two herb teas were analysed in duplicate for prenylflavonoids (Table 4). The data show a large degree of quantitative variation, from an absence of prenylflavonoids in beer no. 4 to a total of 4.0 mg/l in beer No. 7. ‘‘Zero-flavonoid’’ beer No. 4 served as a convenient matrix for the above accuracy experiments. Since hops are the only source of prenylflavonoids in beer, it is not a surprise that there is a relationship between hopping rate or bitterness and prenylflavonoid content. Compare, for instance, the rather mellow US major brand beers (average total of 0.68 mg/l) with the reputedly bitter beers 5, 6 and 8 (average total content of 3.2 mg/l). The prenylflavonoid patterns are similar in most beers with isoxanthohumol, xanthohumol and 6-prenylnaringenin as the most important prenylflavonoids in terms of content (>90% of the total). These compounds are accompanied by minor amounts of 8-prenylnaringenin and 6-geranylnaringenin. Desmethylxanthohumol was detected in only one of the beers and only in a trace amount. Beer No. 5 is characterized by a relatively high xanthohumol to isoxanthohumol ratio (0.52 versus 0.04 on average in the US major brands). This indicates a shorter period of exposure of hops to boiling wort in the brew kettle (during which the isomerization rate is fastest) or secondary addition of hops near the end of wort boiling.

The absence of prenylflavonoids in beer No. 4 and the low levels in beer No. 10 (Table 4) may be

Table 4
Prenylflavonoid contents in beers and herb teas, mg/l (C.V., $n=2$)

No.	Beer type	Xanthohumol	Isoxanthohumol	8-Prenylnaringenin	6-Prenylnaringenin	6-Geranylnaringenin	Total
US major brands							
1	Lager/pilsner	0.034 (4.0)	0.50 (1.3)	0.013 (1.7)	0.034 (9.2)	0.011 (2.3)	0.59
2	Lager/pilsner	0.009 (1.8)	0.68 (0.1)	0.014 (4.2)	0.038 (1.0)	0.006 (0.1)	0.75
3	Lager/pilsner	0.014 (4.1)	0.40 (3.0)	0.017 (0.3)	0.031 (1.4)	0.005 (1.4)	0.46
4	Lager/pilsner	–	–	–	–	–	–
Northwest/US microbrews							
5	Am. porter	0.69 (1.1)	1.33 (3.8)	0.24 (0.7)	0.56(2.2)	0.074 (7.9)	2.90
6	Am. hefeweizen	0.005 (0.9)	0.30 (0.2)	0.008 (2.5)	0.011 (2.1)	0.001 (2.1)	0.33
7	Strong ale	0.24 (0.2)	3.44 (1.5)	0.11 (0.5)	0.20(0.3)	0.006 (15)	4.00
8	India pale ale[a]	0.16 (3.6)	0.80 (8.3)	0.039 (5.2)	0.146 (2.4)	0.016 (8.0)	
Imported beers							
9	Imported stout	0.34 (2.4)	2.10 (0.4)	0.069 (2.2)	0.17(0.7)	0.007 (6.8)	2.68
10	Imported lager	0.002 (15)	0.04 (3.2)	0.001 (141)	0.001(28)	–	0.04
11	Imported pilsner	0.028 (8.8)	0.57 (1.2)	0.021 (1.8)	0.055 (2.0)	0.007 (6.3)	0.68
12	Imported pilsner	0.012 (3.5)	1.06 (0.3)	0.008 (4.0)	0.022 (7.7)	0.001 (16)	
Other beverages							
1	Non-alcohol beer	0.003 (14)	0.11 (1.9)	0.003 (9.2)	0.007 (8.2)	–	0.12
2	Herb tea	0.004 (18)	0.009 (3.7)	0.002 (2.7)	0.004 (5.4)	–	0.02
3	Herb tea	–	–	–	–	–	–

[a] Trace of desmethylxanthohumol also present.

attributed to the use of CO_2 extracts of hops in the brewing process to add bitterness to the beer. Unlike the bitter acids, xanthohumol and related prenylflavonoids are virtually not extracted with CO_2. The amount of xanthohumol found in a commercial supercritical CO_2 extract, 115 mg/kg extract, was equivalent to ca. 1% extraction yield from hop cones having an average xanthohumol content.

The two herb teas examined are very low in prenylflavonoids in comparison with the hopped beers. The tea bags contained about 2 g of a mixture of dried herbs. To account for prenylflavonoid concentrations in the range 0–20 μg/l in the teas (Table 4), the herb mixture must contain only minor amounts of hops. Decay of xanthohumol during storage of hops may also offer an explanation for the low levels in the herb teas. Hänsel and Schulz [10] found a decrease in xanthohumol content of about 80% in hops that had been stored for over four years.

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