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Fast Screening Method to Determine Hop's Phytoestrogens in Beer

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Abstract A novel, fast, and simple method to determine three phytoestrogens [xanthohumol (XN), isoxanthohumol (IXN), and 8-prenylnaringenin (8PN)] in beer samples by high-performance liquid chromatography with diode-array detection has been developed. The selected method involves purification by solid phase extraction and separation achieved on a C_{18} column by using a gradient elution program. This consists of a mixture of acetonitrile, water, and methanol (all of them with 1% of acetic acid) at a flow ratio of 1 mL/min. The detection wavelengths were set at 370 nm for XN and 280 nm for IXN and 8PN. Average analyte recoveries were higher than 75% with relative standard deviations lower than 5%. The detection limits were between 0.01 and 0.08 mg/L, and the quantitation limits were between 0.02 and 0.15 mg/L. The method has been validated and applied to the analysis of several types of commercial beers obtained from local markets, where results showed the presence of IXN and 8PN in most of the samples and the absence of XN. Thus, the presented method gives to brewing chemists a validated, reliable, fast, and affordable analytical tool to identify and quantify the three most important hop's prenylflavonoids in beer.

Keywords Beer · HPLC-DAD · Phytoestrogens

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Introduction

Hop (*Humulus lupulus* L) has long traditionally been used for medicinal purposes, for the treatment of sleeping and nervous disorders, as mild sedative, and for the activation of gastric function as bitter stomachic; moreover, from an ethnographic point of view, hop has been used worldwide including Native American tribes, European, Indian and Chinese cultures, and from prehistoric periods (Zanoli and Zavatti 2008). However, in recent years, hop-derived products such as beers and herbal products have gained much attention due to arising new pharmacological properties such as estrogenic activity (Zanoli and Zavatti 2008; Milligan et al. 1999; Stevens and Page 2004) and cancer-related bioactivities (Gerhäuser 2005a; Colgate et al. 2007; Koo et al. 2008), among others (Zanoli and Zavatti 2008; Mendes et al. 2008; Yang et al. 2008; Choi et al. 2009).

The aforementioned properties are based on a particular class of secondary metabolites known as prenylflavonoids, which structurally differs from those traditionally associated with hop such as terpenoids, α-acids and β-acids, catechins, and flavonol glycosides (Stevens and Page 2004). Prenylflavonoids are a minor class of flavonoids characterized by a prenyl side chain attached to the aromatic benzopyrene skeleton (Heide 2009) and have a relatively narrow distribution in the plant kingdom (Botta et al. 2005). This prenyl moiety increases the lipofilicity and

confers to the flavonoid molecule a strong affinity to biological membranes, displaying a series of interesting biological activities (Barron et al. 1996).

Hop, which is mostly used in the brewing industry to add bitterness and aroma to beer, is gifted by nature with a generous collection of prenylflavonoids (Zanoli and Zavatti 2008). The two most important hop's prenylflavonoids are xanthohumol (XN) and 8-prenylnaringenin (8PN), although more than 15 prenylchalcones and prenylflavanones are found in it (Zanoli and Zavatti 2008; Stevens and Page 2004). Xanthohumol exerts in vitro a wide range of interesting biological properties that may have therapeutic utility to inhibit HIV-1 (Wang et al. 2004), the replication of *Plasmodium falciparum* (Gerhäuser 2005b), and to treat atherosclerosis (Stevens et al. 2003), and it is classified as a potential anticancer and a cancer prevention agent (Colgate et al. 2007; Gerhäuser et al. 2002; Vanhoecke et al. 2005; Guerreiro et al. 2007). On the other hand, 8-prenylnaringenin has been found to be the most potent phytoestrogen described until now (Zanoli and Zavatti 2008). The therapeutic properties of these compounds have led to the use of these ones to obtain functional products such as herbal supplements or fortified foods such as, for example, xanthohumol-enriched beer (Magalhaes et al. 2008).

However, the amount of active prenylflavonoids present in a nutraceutical or in a functional food, i.e., enriched beer, changes due to phenological or varietal oscillations, herbal supplements; and the effect of processing for the beer itself. For instance, wort boiling process and the form in which hop bitterness and aroma is added to beer have an influence onto the prenylflavonoid content (Magalhaes et al. 2008, 2007).

Therefore, to detect the presence of these biologically active chemicals in hop-containing products, such as beers, herbal products, or hop extracts, it seems necessary to have reliable, fast, and easy-to-use analytical techniques accommodated to a factory–production environment. The most common separation technique to analyze flavonoids in foods, drinks, and other biological samples is reversed-phase high-performance liquid chromatography (HPLC) with UV, electrochemical, or evaporate light-scattering detection. Furthermore, gas chromatography and electromigration techniques, such as capillary zone electrophoresis or capillary electrochromatography, can be employed as well (Molnár-Perl and Füzfai 2005). To analyze hops and beer samples, the coupling of HPLC with mass spectrometry (MS) using electrospray ionization or atmospheric pressure chemical ionization in both polarity modes is vastly reported in the literature (Magalhaes et al. 2007, 2008, 2010; Rong et al. 2000; Stevens et al. 1999; Maragou et al. 2008). On the other hand, easy-to-use methods based in simple HPLC–UV for the detection of hop's prenylflavonoids have not been so extensively developed; only one work devoted to the analysis of XN in beer based on cloud point extraction by using HPLC–UV has been recently published (Chen et al. 2010), but isoxanthohumol (IXN) and 8PN were not studied in this paper. When other approaches have been applied, they have focused on phenols and common flavonoids, i.e., not prenylated (Alonso-García et al. 2004), may be consequence of their own chemical nature. Prenylated flavonoids are found in hop at a very low concentration. Furthermore, the high amount of related compounds found in hop, together with the drastic change in polarity, due to the prenylated side chain, that lead to an abnormal chromatographic behavior, when compared with non-prenylated ones, may become cumbersome and the development of simple HPLC techniques for their analysis is difficult.

Therefore, in this paper, an easy-to-use, fast, accurate, and validated high-performance liquid chromatography with diode-array detection (HPLC–DAD) method is presented for the detection of the most abundant hop's prenylated flavonoids, showing some advantages compared to those actually available methods. Finally, the method is applied to the analysis of commercial beers.

Material and Methods

Reagents and Chemicals

Xanthohumol (XN) was supplied by Sigma-Aldrich Chemie GmbH (Steinheim, Germany), isoxanthohumol (IXN) and 8-prenylaringenin (8PN) were purchased from Alexis biochemical (Lausen, Switzerland). Analytical-grade dimethyl sulfoxide (DMSO) or HPLC-grade acetonitrile and methanol were obtained from Labscan Ltd. (Dublin, Ireland). Glacial acetic acid was purchased from Panreac Química S.A. (Barcelona, Spain). Sep-Pak Cartridges C_{18} 360 mg 55–105 μm were from Waters Corporation (Milford, MA, USA). Ultrapure water was obtained in a Milli-RO plus system together with a Milli-Q system from Millipore (Bedford, MA, USA). All the solvents and solutions were passed through a 0.45-μm nylon filter from Phenomenex (Torrance, CA, USA) before use. Ultrasonic bath Bransonic 5 was obtained from Scharlau Chemie S.A. (Barcelona, Spain). Syringe filters (17 mm nylon 0.45 μm) from Nalgene (Rochester, NY, USA) were used.

Preparation of Standard Solutions

Stock solutions of the three phytoestrogens were prepared in DMSO at a concentration of 1,000 mg/L. Aqueous solvent was avoided because it has been reported that XN was not stable in aqueous solutions (Stevens et al. 1999).

Working solutions of pertinent concentrations were made daily by an appropriate combination and a serial dilution of standard solutions with DMSO. All standards and stock solutions were kept in darkness at +4 °C and they were stable for over 1 month.

Instruments and Conditions

An Agilent Technologies (Palo Alto, CA, USA) 1100 series HPLC system consisting of a vacuum degasser, a quaternary solvent pump, an autosampler with an external column oven, and a diode array detector (DAD) with scanning capabilities, all of them controlled by a Chemstation software, was used.

An ODS2 Spherisorb C_{18} 80 Å 5 μm (250×4.0 mm i.d.) from Waters (Milford, MA, USA) was used as an analytical column for LC separation. The mobile phase consisted of (1) 1% acetic acid in acetonitrile, (2) 1% acetic acid in water, and (3) 1% acetic acid in methanol in a gradient elution analysis programmed as follows: 0 min, 44% (2), 0% (3); 0–10 min, 51% (2), 5% (3); 10–12 min, 44% (2), 0% (3); with 5 min of post-time at a flow rate of 1 mL/min. The column temperature was set at 25 °C and the injection volume was 20 μL. The detection wavelengths were set at 370 nm for XN and 280 nm for IXN and 8PN, respectively.

Beer Samples

Different types of commercial beer samples were purchased from local markets of Madrid (Spain). They differed in the origin, color, and alcoholic grade to cover a wide range of beers types: three of them were of pale lager type, two of them were of dark type, and one of them was an alcohol-free beer.

Sample Preparation

After the optimization study described below, the next conditions were selected: the beer samples were degassed and afterwards sonicated for 1 min to remove the foam, and passed through a 0.45-μm nylon filter. Two milliliters of the beer sample was taken and loaded onto a Sep-Pak cartridge, which was previously preconditioned with 2 mL of acetonitrile. Then, the cartridge was washed with 2 mL of water, and the compounds were eluted with 2 mL of acetonitrile. After filtering, an aliquot of 20 μL was injected onto the HPLC–DAD system.

Statistical Analysis of Data

As the aim of this work is to develop a simple method to determine hop's phytoestrogens, basic but efficient chemometric statistical tools from Excel (Microsoft Office 2007, Microsoft Corporation, Redmond, WA, USA) have been employed to analyze the obtained data.

Parameters for the linearity studies and the confidence intervals were calculated by using the regression and analysis of variance tools from Excel. A Student's t test was made to verify that the calibration curve followed a linear trend. The $t_{\text{experimental}}$ must be higher than the $t_{\text{tabulated}}$ which was the t_{student} calculated for $n-2°$ of freedom and a probability of 0.95. For all the cases, it was found that $t_{\text{experimental}} > t_{\text{tabulated}}$, so it could be said that the trend was linear. Finally, an analysis of variance was made with the raw data from the precision and recovery studies; the results revealed the absence of significant differences between the assayed levels of spiking.

In all experiments, P values of <0.05 were considered to indicate statistical significance.

Results and Discussion

Sample Treatment

Beer samples were degassed by gentle swirling in a beaker followed by 1-min sonication. Beer samples were then filtered by using 0.45-μm nylon filters.

Before loading the beer onto the Sep-Pak cartridges, they were preconditioned; different volumes of acetonitrile were tested to precondition the cartridge (1–4 mL); it was found that 2 mL of acetonitrile was enough to precondition the cartridge. The beer sample volume was also studied; for this purpose, different volumes of beer (1–4 mL) were loaded onto the cartridge. The recoveries were almost constant up to 2 mL, decreasing for higher volumes, so, a beer volume of 2 mL was chosen. As beer contains other substances apart from the studied compounds, the direct elution of the cartridges generated dirtier chromatograms, and there were some interferences which affected the correct detection of the phytoestrogens. To avoid these problems, it was necessary to wash the cartridges. To that purpose, several volumes of water (1–4 mL) were tested. The results showed that 2 mL of water was enough to obtain cleaner chromatograms and to suppress the interferences. Finally, the volume of acetonitrile to elute the XN, IXN, and 8PN was studied. The phytoestrogens were eluted with different acetonitrile volumes (1–4 mL), and it was found that the elution with 2 mL acetonitrile was good enough to obtain recoveries higher than 75% for all the compounds. And then, an aliquot of 20 μL of this extract was injected onto the HPLC system.

It should be noted that the selected sample treatment would be done in 10 min, which is ≈40% faster than the procedure proposed for determining XN in beer by HPLC–UV (Chen et al. 2010). The recovery percentages were

between 75% and 98% (Table 1) for the different concentrations and compounds assayed; again, the results were consistent with those previously published with more time-consuming sample treatments and more expensive detectors.

Chromatographic Conditions

To achieve a good separation between the three compounds, several tests were done. We started with the conditions proposed by Stevens et al. (Stevens et al. 1999) who employed a binary gradient with acetonitrile and 1% formic acid in water on a RP C_{18} column to separate XN and related prenylflavonoids. These conditions did not allow us to obtain a good separation between the three compounds. It must be remarked that although they were tested on columns of different lengths (100, 150, and 250 mm), a ODS2 Spherisorb C18 (250 mm) column was selected because with shorter columns, it was not possible to obtain the full separation of the IXN and 8PN peaks from some peaks attributed to the matrix. To solve this problem, different gradient elution programs were tested, but none allowed the separation in a shorter period of time. As one of the goals was to separate all the compounds in a short time as possible, we decided to change the formic acid to acetic acid and add this acid (1% *v*/*v*) to the acetonitrile. With this new mobile phase, the analysis time was shortened, but IXN eluted first, coeluting with a matrix component.

Then, we added a new component to the mobile phase, methanol with 1% of acetic acid, to solve this problem. After several tests, we were able to separate all the compounds in less than 10 min with a mobile phase which consisted of (1) 1% acetic acid in acetonitrile, (2) 1% acetic acid in water, and (3) 1% acetic acid in methanol in a gradient elution analysis programmed as follows: 0 min, 44% (2), 0% (3); 0–10 min, 51% (2), 5% (3); 10–12 min, 44% (2), 0% (3); with 5 min of post-time at a flow rate of 1 mL/min.

Selection of the Detection Wavelength

The absorbance spectra in the UV region for the three compounds were checked to select the optimal wavelength of detection. IXN and 8PN presented an absorbance maximum at 280 nm while XN showed high absorbance levels at this wavelength, being its maximum at 370 nm. In order to obtain the highest response for each of the compounds, two different wavelengths were selected: 270 nm for IXN and 8PN and 370 nm for XN.

Temperature

No significant variation was observed on the retention times and peak symmetries according to the column temperature changes (20–40 °C), room temperature (25 °C) was finally chosen.

Injection Volume

Although the main target of this work was to develop a fast screening method to determine XN, IXN, and 8PN in beer samples, the possibility of enhancing the detection limits by injecting high sample volumes was considered. In the established conditions, volumes from 10 to 40 μL of a standard solution of 0.5 mg/L were injected; the obtained results showed that, for injection volumes higher than 20 μL, the chromatographic peaks began to be somewhat deformed and the signal-to-noise (S/N) ratio did not improve. Therefore, an injection volume of 20 μl was adopted as optimum.

The proposed chromatographic conditions generated narrow and reproducible chromatographic peaks. As an

Table 1 Precision and recovery studies

Compound	Spiked level (mg/L)	Precision studies (n=5)		Recovery studies (n=5)	
		Repeatability RSD (%)	Intermediate precision RSD (%)	Recovery (%)	RSD (%)
XN	0.05	2.7	2.8	98.0	2.3
	0.50	2.4	2.5	94.5	2.1
	4.80	2.3	2.4	96.8	1.9
IXN	0.12	2.9	3.3	86.7	3.1
	0.56	4.1	2.9	92.4	3.4
	4.76	3.7	3.2	89.4	2.9
8PN	0.40	3.1	2.7	75.2	2.8
	1.30	2.5	2.3	80.9	2.5
	4.90	2.9	3.1	82.1	3.2

example of this statement, chromatograms of different beer samples, non-spiked and spiked with IXN, 8PN, and XN can be seen in Fig. 1.

Validation of the Method

Validation was carried out following the ICH guidelines (1996) and IUPAC technical report of 2002 (Thompson et al. 2002) determining selectivity, limits of detection and quantitation, linearity, precision, and trueness.

We studied the selectivity by injecting extracts of non-spiked beer samples. As we do not have a real blank of beer (without IXN and 8PN), we checked the purity of the peaks assigned to each phytoestrogens by comparing the absorbance spectra of the beer and standard peaks. So, as can be seen in Fig. 1, the ability of the method to separate XN, IXN, and 8PN in less than 10 min has been demonstrated, and also, the purity of each peak of interest has been confirmed since they do not coelute with any other matrix compound. These results matched with those ones obtained with the purity tool option included in the Agilent Chemstation software.

The detection limit (LOD) and quantitation limit (LOQ) were determined by injecting a number of extracts of beer samples (n=20) and measuring the magnitude of the background analytical response at the retention time of

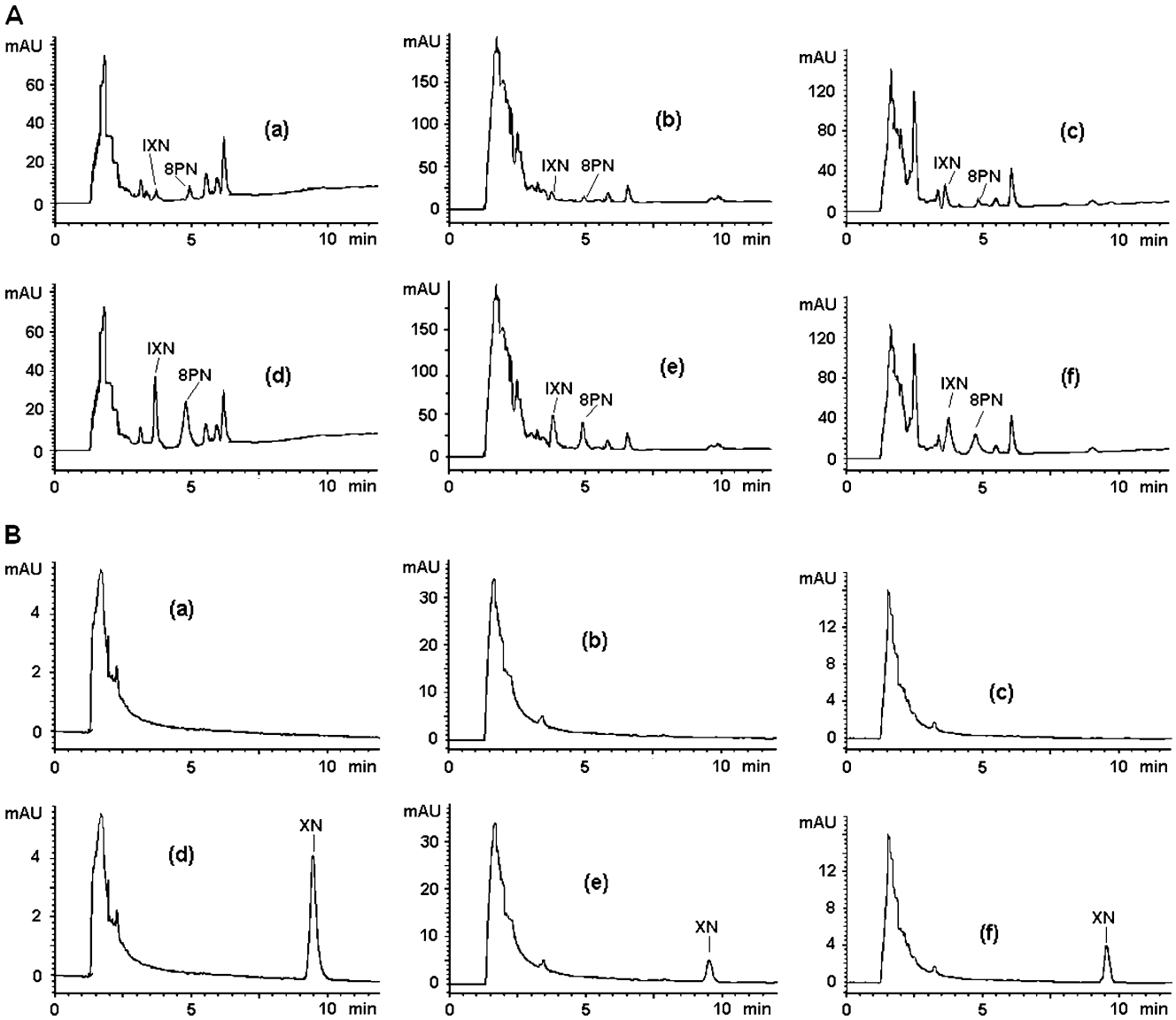

Fig. 1 **a** HPLC–DAD chromatograms obtained with a ODS2 Spherisorb C_{18} chromatographic column at 280 nm of a non-spiked and spiked with IXN and 8PN at 1 mg/L (*a*, *d*) alcohol free, (*b*, *e*) dark and (*e*, *f*) pale (golden) beer samples. **b** HPLC–DAD chromatograms at 370 nm of a non-spiked and spiked with XN at 0.15 mg/L (*a*, *d*) alcohol free, (*b*, *e*) dark and (*e*, *f*) pale (golden) beer samples. The chromatographic conditions are described in detail in the text of the manuscript (Instrument and Conditions Section)

Table 2 Linearity studies (n=5) for the three phytoestrogens

Compound		Analytical range (mg/L)	R^2 (sy/x)	a ± s_a	b ± s_b
XN	Standard	0.02–10	0.998 (56.7)	−67.5±30.3	77.9±2.7
	Beer 1	0.02–10	0.997 (75.3)	−106.3±63.2	79.5±5.1
	Beer 2	0.02–10	0.998 (69.7)	−82.7±51.7	76.9±4.6
	Beer 3	0.02–10	0.993 (62.3)	−75.5±61.8	79.2±5.5
	Beer 4	0.02–10	0.995 (59.8)	−90.3±59.3	81.5±5.3
	Beer 5	0.02–10	0.994 (69.49)	−63.5±50.1	75.4±4.5
	Beer 6	0.02–10	0.996 (70.7)	−66.7±53.7	78.3±4.1
IXN	Standard	0.05–10	0.999 (32.6)	−36.1±18.1	12.1±0.4
	Beer 1	0.05–10	0.997 (54.4)	4.1±3.5	12.6±0.8
	Beer 2	0.05–10	0.996 (45.2)	6.4±2.9	12.5±0.6
	Beer 3	0.05–10	0.998 (39.3)	7.2±2.5	11.4±1.0
	Beer 4	0.05–10	0.996 (42.9)	4.4±2.3	12.1±0.5
	Beer 5	0.05–10	0.997 (46.3)	8.7±3.6	12.0±0.6
	Beer 6	0.05–10	0.998 (41.9)	1.0±3.2	12.1±0.7
8PN	Standard	0.15–10	0.999 (3.7)	−14.1±2.7	2.5±0.1
	Beer 1	0.15–10	0.998 (4.4)	0.6±0.2	2.5±0.3
	Beer 2	0.15–10	0.997 (2.8)	−3.6±0.2	2.3±0.2
	Beer 3	0.15–10	0.998 (4.6)	0.4±0.1	2.6±0.4
	Beer 4	0.15–10	0.997 (3.0)	−4.2±0.3	2.4±0.2
	Beer 5	0.15–10	0.999 (4.7)	0.7±0.1	2.6±0.3
	Beer 6	0.15–10	0.997 (3.8)	0.9±0.2	2.5±0.4

Beers 1–2 (dark), beers 3–5 (golden/pale), beer 6 non-alcohol

sy/x standard deviation of the vertical distances of the points from the line, *a* intercept with the *y* axis, s_a standard deviation of the intercept, *b* slope of the line, s_b standard deviation of the slope

XN because it was not present in the samples; for IXN and 8PN, since all beer samples contain them, the noise was measured as close as possible to their peaks. We estimated experimentally the LOD and LOQ as three and ten times the S/N. The obtained values for LOD and LOQ were 0.01 and 0.02 mg/L for XN, 0.02 and 0.05 mg/L for IXN, and 0.06 and 0.15 mg/L for 8PN, respectively, which were found to be consistent with those described in the literature for these compounds and other flavonoids analyzed with similar methods (Maragou et al. 2008; Chen et al. 2010).

Matrix-matched calibration standard curves were used to quantify the phytoestrogens in beer. Beer samples were spiked with variable amounts of the three compounds (Table 2) for a different analytical range depending on the compounds' LOQ, and all the samples were treated according to the procedure described above. Those spiked beer samples were analyzed with the method previously described.

The obtained extracts were considered as standards to get the calibration graphs. Concentration versus the area was plotted to prepare the matrix-matched calibration curves of each individual set of standard series. To obtain the concentration of IXN and 8PN, as they were present in all the beer samples, the standard addition method was employed. Determination coefficient values (R^2) as good as 0.99 (or better) were obtained, as seen in Table 2.

Quantitation for XN could be done with a standard calibration curve since it is not necessary to use matrix-matched calibration as the slopes of the matrix-matched and standard calibration curves were within the same range of 70.5–85.5 (confidence interval obtained from the analysis of the calibration curves of Table 2), $p<0.05$.

For IXN and 8PN, it would be necessary to employ a matrix-matched calibration curve (standard addition method) for the six beer samples. Although the slopes were within the same range for the standard and matrix-matched calibration curves (11.0 to 13.2 and 2.2 to 2.9) for IXN and 8PN, respectively, the intercept value logically differed between the standard and the matrix-matched calibration curves, as IXN and 8PN were in the studied beers. For high concentrations, where area values are high, this might not be a problem, considering the contribution of each term into the equation. But, for lower concentrations, to quantify with

Table 3 Concentrations (mg/L) of XN, IXN, and 8PN found in beer samples (%RSD, n=3)

Sample	XN	IXN	8PN
Beer 1	<LOD	0.32 (1.7)	0.16 (1.1)
Beer 2	<LOD	0.53 (2.5)	<LOD
Beer 3	<LOD	0.62 (1.4)	0.19 (2.3)
Beer 4	<LOD	0.37 (2.6)	<LOD
Beer 5	<LOD	0.71 (1.2)	0.18 (2.8)
Beer 6	<LOD	0.08 (1.5)	0.31 (2.1)

Beers 1–2 (dark); beers 3–5 (golden/pale), beer 6 non-alcohol

a standard curve will lead to an important error in the measurement of IXN and 8PN, even for lower 8PN concentrations. When an accurate concentration value is needed, its own calibration curve should be used.

The same analyst evaluated the precision. The results of repeatability and intermediate precision experiments are shown in Table 1. Three different concentrations were assayed for each compound (low, medium, and high, $n=5$). The method was found to be precise as the relative standard deviation (RSD) values for repeatability and intermediate precision studies were lower than 5% in all the cases.

Recovery and trueness were determined on spiked beer samples at the three different concentration levels ($n=5$). Table 1 shows the obtained results. The mean recoveries ranged from 75% to 98%, being the highest recoveries for XN and the lowest for 8PN. As it can be observed, there were no differences among the three concentrations studied for each compound. It could be also remarked that all the percentage RSD values were <5%.

Application of the Method

The developed method was applied to the analysis of the three phytoestrogens in several commercial beer samples. As could be seen in Table 3, the content of IXN and 8PN did not seem to follow any pattern according to the obtained results; so, it could be stated that the type of beer and the origin in the analyzed samples do not possess a direct influence onto the presence of these compounds in beer. Meanwhile, the alcoholic content showed an influence onto the presence of IXN according to the obtained value (of all the studied beers, it was lower in Beer 6), but there was no relationship with 8PN content, which was higher compared with the other beer samples. It must be pointed out that although the content of 8PN in the analyzed beers is a bit higher in comparison with the results presented in some works, it is in the range of previously published data in beer (Stevens et al. 1999). Meanwhile, the measured content of IXN fits perfectly with the data shown in the existing literature.

The absence of XN, the major hop prenylflavonoid, is remarkable, but it has been previously observed by Magalhaes et al. (2008) and can be explained by a transformation of most XN into IXN during wort boiling. The predictable absence of this compound in most beers leads to missing the functional properties attributed to XN, present in hop. However, the finding that 8PN is relatively more abundant in the non-alcohol beer than in the others is noteworthy. Thus, in alcohol-free beer, 8PN represents more than 90% of the detected hop prenylflavonoids, much more than in any of the other analyzed beers. It is estimated that only a third of the individuals are able, through their colonic microflora, to convert the inactive IXN into the active 8PN (Possemiers et al. 2008). Therefore, alcohol-free beer could be a good nutritional source of this phytoestrogen.

However, whether the higher relative abundance in alcohol-free beer is due to the fabrication process or to the solvent properties of the beer itself due to the lack of ethanol is still unclear and, at present, it is under research.

Concluding Remarks

The new validated HPLC–DAD methods is fast (with a running time below 10 min and a total spent time below 20 min), simple, precise, and easy to use for the determination of XN, IXN, and 8PN in beer samples.

Solid phase extraction avoids interferences from matrix and provides sample preparation lasting for less than 10 min. The quantization of XN can be done with the standard curve, while for IXN and 8PN, the use of a matrix-matched one is needed.

In comparison with the described methods HPLC–DAD and LC–MS, an excellent separation and similar LOD and LOQ to those described for some of these compounds and other beer flavonoids analyzed by HPLC–UV was obtained.

The method has proved to be precise and repeatable, RSD values lower than 5%, with recoveries ranged between 75% and 98%, depending on the compound.

Finally, and regarding the application of the method, the results of the analysis were consistent with the amounts of these prenylflavonoids described elsewhere. The method may be of high utility in environments where short time responses are needed, such as factories or fast screening studies; being a cheaper, friendly, and affordable alternative to the existing HPLC–MS methods.

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