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Analysis of Hop Acids and Their Oxidized Derivatives and Iso-α-acids in Beer by Capillary Electrophoresis–Electrospray Ionization Mass Spectrometry

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This study investigates the applicability of on-line coupling of capillary electrophoresis with electrospray ionization tandem mass spectrometry (CZE-ESI-MS) for the separation and characterization of α- and β-acids and oxidized hop acids from crude extracts of different hop varieties. CZE-ESI-MS with negative-ion electrospray ionization proved to be a suitable technique for the determination of these types of natural compounds and their oxidized derivatives. The CZE parameters (pH, concentration, and buffer type) and ESI-MS parameters (nature and flow rate of the sheath liquid, nebulizer pressure, drying gas flow rate, temperature, and compound stability) were optimized. The optimized method provides the potential for a fast qualitative determination of hop acids and their oxidation compounds. The method was also applied to the determination of iso-α-acids in beer.

KEYWORDS: Acetone hop extract; hop acids; iso-α-acids; capillary zone electrophoresis; mass spectrometry

INTRODUCTION

Extracts of hop cones, the female flowers of *Humulus lupulus* L., are used for adding aroma and flavor in the beer-brewing process. Hops contain hundreds of components but of particular interest are the so-called resins, containing mainly hop acids, hop oil, and polyphenols. These three classes of resin are important as biochemical markers to differentiate hop varieties. The hop acids, part of the soft resin fraction, consist of two related series, the α-acids (humulone, cohumulone, and adhumulone) and the β-acids (lupulone, colupulone, and adlupulone) (*1*). Besides the two series of normal-, co-, and ad-homologues there are also some minor hop acids in the plant, including posthumulone/postlupulone, prehumulone/prelupulone and adprehumulone (*2*). These are present as a complex mixture of varying composition and concentrations. The relative proportions of α-acids and β-acids as well as the content of co-homologues depend on the hop variety and, for any given variety, on the growing conditions.

Many different varieties of the *H. lupulus* L. species exist, each one with its own different composition and agronomic characteristics. Traditionally, hop varieties have been classified into two groups, namely, "aroma" or "bitter" types, depending on their α-acid content and flavor characteristics.

Hops are prone to oxidation and chemical deterioration. Both the α- and β-acids are very susceptible to oxidation and degradation during storage. Their oxidation products affect beer flavor significantly because once α-acids have been oxidized, they can no longer be isomerized into iso-α-acids; thus, the hops' bittering potential decreases, and the aroma becomes unpleasant and "cheesy". It is also important to package the hops properly, which involves keeping them in refrigerated storage at temperatures of between 0 and 5 °C, removing as much oxygen as possible, and storing them in an oxygen barrier material (*3*, *4*). Apart from the storage conditions, each variety has a particular tendency to be oxidized, and so the oxidation state of hops is an important quality factor that needs to be looked at closely for quality control in the brewing industry.

During the brewing process the virtually insoluble α-acids of the hop extract are converted into the more soluble iso-α-acids, which give the typical bitter taste to the beer. In addition to imparting bitter taste, iso-α-acids exhibit other interesting features: they have tensioactive properties, thereby stabilizing the beer foam, and they inhibit the growth of Gram-positive bacteria.

An analysis of the hop acids in hops is important for quality control, and many methods have been developed to provide a

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quantitative analysis of the α-acids in hops and hop products. A widely used empirical method extracts the bitter components into solvents and measures them quantitatively by spectrophotometry. HPLC with UV detection is also routinely used to analyze bitter acids (*5*−*12*). Nevertheless, UV is neither sensitive nor selective enough for the direct identification of minor hop acids in complex mixtures. The instability and structural similarity of the bitter hop acids cause difficulty in routine analysis. Recently, the detection of these compounds by HPLC coupled to mass spectrometry was investigated. The six major bitter hop acids have been analyzed by HPLC coupled with atmospheric pressure ionization tandem mass spectrometry (APCI-MS-MS) (*2*) and with negative electrospray ionization mass spectrometry (*13*).

Other techniques such as capillary electrophoresis (CE) in its different modes have been applied to the analysis of hop acids, that is, capillary zone electrophoresis (CZE) (*14*), micellar electrokinetic chromatography (MEKC) (*15*, *16*), and microemulsion electrokinetic chromatography (MEEKC) (*17*−*19*), using a UV detector. The iso-α-acids have also been determined using MEKC-UV (*20*−*23*).

The aim of this work has been to develop the first fast and simple capillary electrophoresis−electrospray ionization mass spectrometry (CE-ESI-MS) method for the identification of hop acids and their oxidation compounds in four varieties of hops: Saaz, Nugget, Magnum, and Columbus. We have also determined iso-α-acids in beer to demonstrate the applicability of this method.

MATERIALS AND METHODS

Chemicals. The hop acid standard, an international calibration extract ICE 2, composed of a mixture of α-acids (34.94% humulone + adhumulone and 14.45% cohumulone) and *β*-acids (12.02% lupulone + adlupulone and 12.92% colupulone), and the iso-α-acid standard ICS-I2, with a mixture of 64.3% *trans*-iso-α-acids (iso-humulone, iso-adhumulone, and iso-cohumulone) were from Labor Veritas, Zürich, Switzerland.

Ammonium acetate, ammonium carbonate, acetic acid, and diethylamine were from Panreac (Barcelona, Spain), ethanolamine and diethanolamine were from Aldrich (Steinheim, Germany), and ammonia was from Merck (Darmstadt, Germany), all of which were used for the CE running buffers at different concentrations and pH values. Buffers were prepared by weighing the quantity indicated in doubly distilled water and adding 2 M ammonium hydroxide to adjust the pH. Triethylamine (TEA) was from Aldrich, and HPLC grade 2-propanol used in the sheath flow, acetone, and sodium hydroxide were from Panreac. All solutions were filtered through 0.45 *μ*m Millipore (Bedford, MA) membrane filters before being injected into the capillary. Distilled water was deionized using a Milli-Q system (Millipore). DSC-Diol and DSC-C18 solid-phase separation (SPE) cartridges were from Supelco (Bellefonte, PA).

Instrumentation. For CE separations we used a P/ACE System MDQ (Beckman Instruments, Fullerton, CA) equipped with a UV−visible detector and a 0−30 kV high-voltage built-in power supply. A bare fused-silica capillary with 50 *μ*m i.d. was from Composite Metal Services (Worcester, U.K.). The detection length to the UV detector was 7 cm, and the total length was 100 cm (corresponding to the MS detection length). The instrument was controlled by a PC running System 32 Karat software from Beckman.

CE was coupled using an electrospray interface (ESI) (model G1607A from Agilent Technologies, Palo Alto, CA) to the MS detector (Bruker Daltonics, Squire 2000). A commercial coaxial sheath-flow interface was used (vide infra). The coaxial sheath liquid and the electrical contact at the electrospray needle tip were delivered by a 74900-00-05 Cole Palmer syringe pump (Vernon Hills, IL). An ESI-MS interface provided both a coaxial sheath liquid makeup flow and a nebulization gas to assist droplet formation. Both the drying gas and the nebulization gas were nitrogen. The mass spectrometer was used in the negative-ion mode, and the capillary voltage was set at 4000 V. The ion trap scanned within the *m*/*z* 300−700 range at 13000 u/s during separation and detection in the scan mode. The maximum accumulation time for the ion trap was set at 5.00 ms, the target count was set at 20000, and the trap drive level was set at 100%. For the connection between the CE system and the electrospray ion source of the mass spectrometer the outlet of the separation capillary was fitted into the electrospray needle of the ion source, and a flow of conductive sheath liquid made electrical contact between the capillary effluent and water for the electrospray needle. The instrument was controlled by a PC running Esquire NT software from Bruker Daltonics.

Before the first use, the uncoated capillaries were conditioned using a rinse with 0.1 M NaOH for 10 min followed by a rinse with water for 5 min and finally a rinse with running buffer for 30 min. Capillary conditioning between runs was carried out by flushing the column for 3 min with water and finally for 5 min with the separation buffer. At the end of the day the capillary was rinsed with water for 30 min and dried for 10 min.

Hop Samples and Beer. Hop pellets of the varieties Saaz and Nugget and bottles of "extra" beer were obtained from the company Grupo Cervezas Alhambra S.L. (Granada, Spain), whereas the varieties Columbus and Magnum were provided by S.A. Española de Fomento del Lúpulo (León, Spain).

It is known that drying temperatures >65 °C cause variable losses of hop acids (*3*), so we induced the oxidation of the α- and *β*-acid standards and four hop varieties: Saaz, Nugget, Columbus, and Magnum. The hop pellets were received in intact, lightproof packaging. Ten grams was reduced to powder with a mortar and heated for 2 h at 80 °C in an oven. We also studied the natural oxidation of Saaz and Nugget by keeping pellets of the harvest of the year 2000 in a plastic vessel at room temperature and in the presence of light for 2 years.

Extraction of Hop Acids and Oxidized Derivatives of the Hop Pellets. To recover the hop acids present in the pellets and their oxidized derivatives, we studied different organic solvents (methanol, ethanol, and acetone) with water (0:100, 25:75, 50:50, 75:25, and 100:0). The extraction protocol was as follows: 2.5 g of hop pellets, previously reduced to powder with a mortar, was extracted three times with 50 mL of acetone/water (75:25 v/v) for 10 min each time. The extracts were combined and brought to dryness in a rotary evaporator under reduced pressure at 60 °C to get rid of any residual solvent. During the last step, 2 mL of acetone/water (50:50 v/v) was added to dissolve the extract, which was then passed through a 0.20 *μ*m membrane filter and analyzed by CE-ESI-MS.

The extraction protocol of the naturally oxidized derivatives and the compounds obtained by induced oxidation was the same for both methods and for all four varieties of hops studied.

Extraction of Isomerized Hop Acids in "Extra" Beer. We assayed two different cartridges, DSC-Diol and DSC-C18, and obtained the best results with DSC-C18. Thus, the subsequent extraction protocol of isomerized hop acids was as follows: a DSC-C18 cartridge placed in a vacuum elution apparatus was conditioned by passing 20 mL of acetone/water (75:25 v/v) and then 10 mL of water through it. Subsequently, 100 mL of degassed "extra" beer was passed through the column. The isomerized hop acids were recovered by passing four portions of 5 mL of acetone/water (75:25 v/v). The final volume was dried in a rotary evaporator under reduced pressure at 60 °C. The residue was reconstituted in 2 mL of acetone/water (50:50 v/v) and passed through a 0.20 *μ*m filter before CE-ESI-MS analysis.

General Procedure. The optimum conditions used for the CE-ESI-MS separation method were as follows: running buffer, 160 mM ammonium carbonate/ammonium hydroxide; pH 9; voltage, 20 kV; 7 s injection time; sheath liquid, 2-propanol/water, 50:50 v/v, with 0.1% TEA delivered at a flow rate of 3 *μ*L/min; drying gas flow rate, 4 L/min at 150 °C; nebulizing gas pressure, 6 psi; and MS analyses carried out using a compound stability of 25%.

RESULTS AND DISCUSSION

Development of the CE-ESI-MS Method. The effects of different separation parameters were studied to obtain the best selectivity, sensitivity, and resolution conditions.

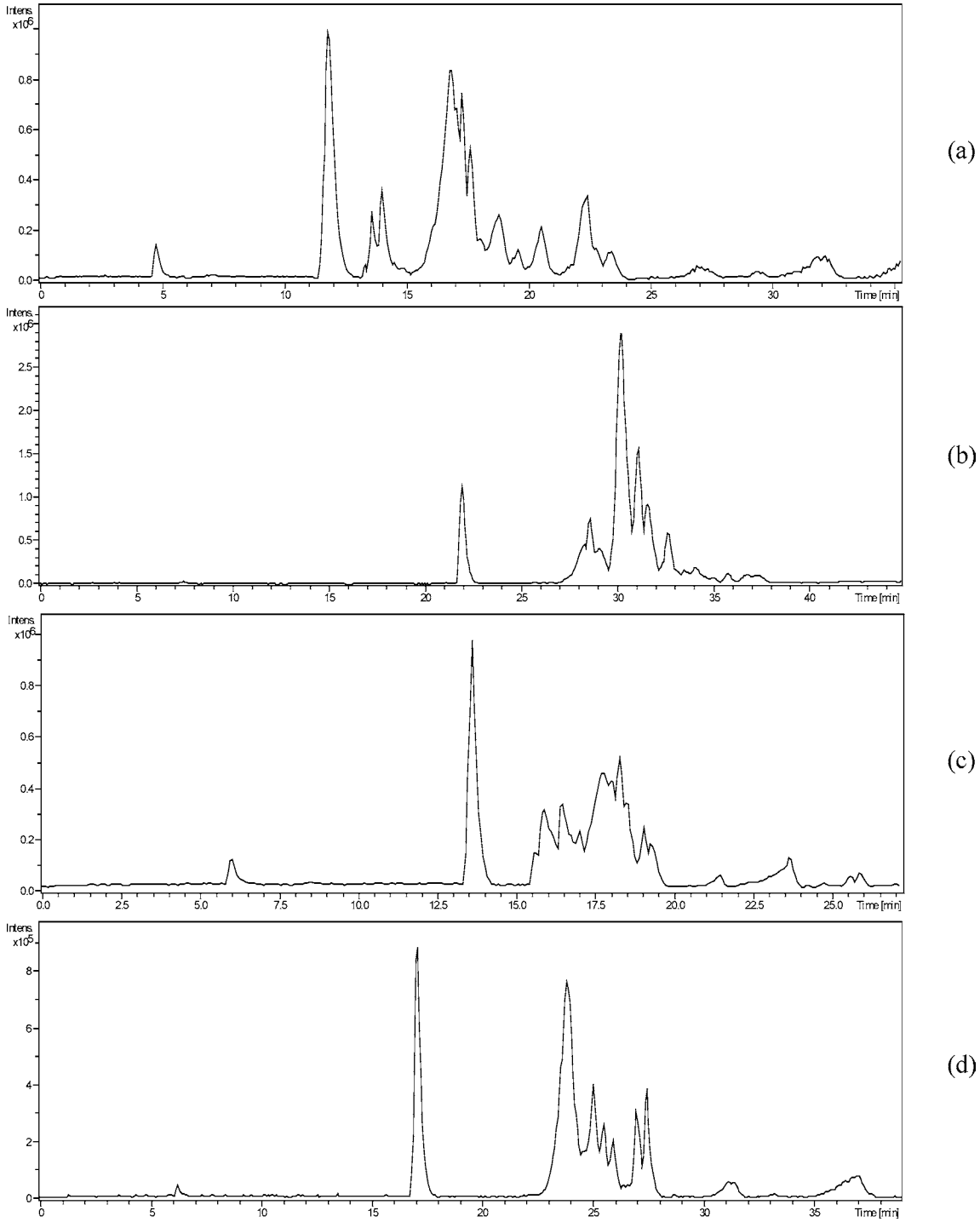

Figure 1. Comparison between different running buffers: (**a**) ammonium carbonate/ammonium hydroxide, 160 mM at pH 9; (**b**) diethylamine/ammonium hydroxide, 500 mM at pH 10.5; (**c**) ammonium hydroxide/acetic acid, 500 mM at pH 9.5; (**d**) ammonium acetate/ammonium hydroxide, 160 mM at pH 10.5. Experimental conditions: 50 μm i.d. fused-silica capillary, 100 cm detector and total length, 20 kV, 7 s of hydrodynamic injection at 0.5 psi; sheath liquid, 2-propanol/water, 50:50 v/v, containing 0.1% TEA; flow rate, 3 μL/min; dry gas, 4 L/min, 150 °C; nebulizing gas pressure, 6 psi. MS analyses were carried out using negative polarity. Compound stability was 25% MS scan *m*/*z* 300–700 (target mass *m*/*z* 550). Sample was oxidized hop pellets of the variety Saaz.

The CE-ESI-MS method was optimized using the extract obtained with 75:25 v/v acetone/water from oxidized Saaz hops because this extract was the most complex and its electropherogram presented the greatest number of peaks. First, the optimum concentration and pH of four volatile running buffers, ammonium carbonate/ammonium hydroxide, diethylamine/ammonium hydroxide, ammonium hydroxide/acetic acid, and ammonium acetate/ammonium hydroxide, were established. The pH of ammonium carbonate/ammonium hydroxide was assayed between 8.5 and 10 at a concentration of 100 mM, and pH 9 showed the best resolution. The concentration was then studied between 100 and 190 mM at pH 9, the best resolution being found with 160 mM.

After studying the influence of pH between 9.5 and 10.5 at a concentration of 100 mM, we chose pH 10.5 as optimum. Diethylamine/ammonium hydroxide was assayed in the range between 100 and 500 mM, and the best resolution was found to occur at a concentration of 500 mM.

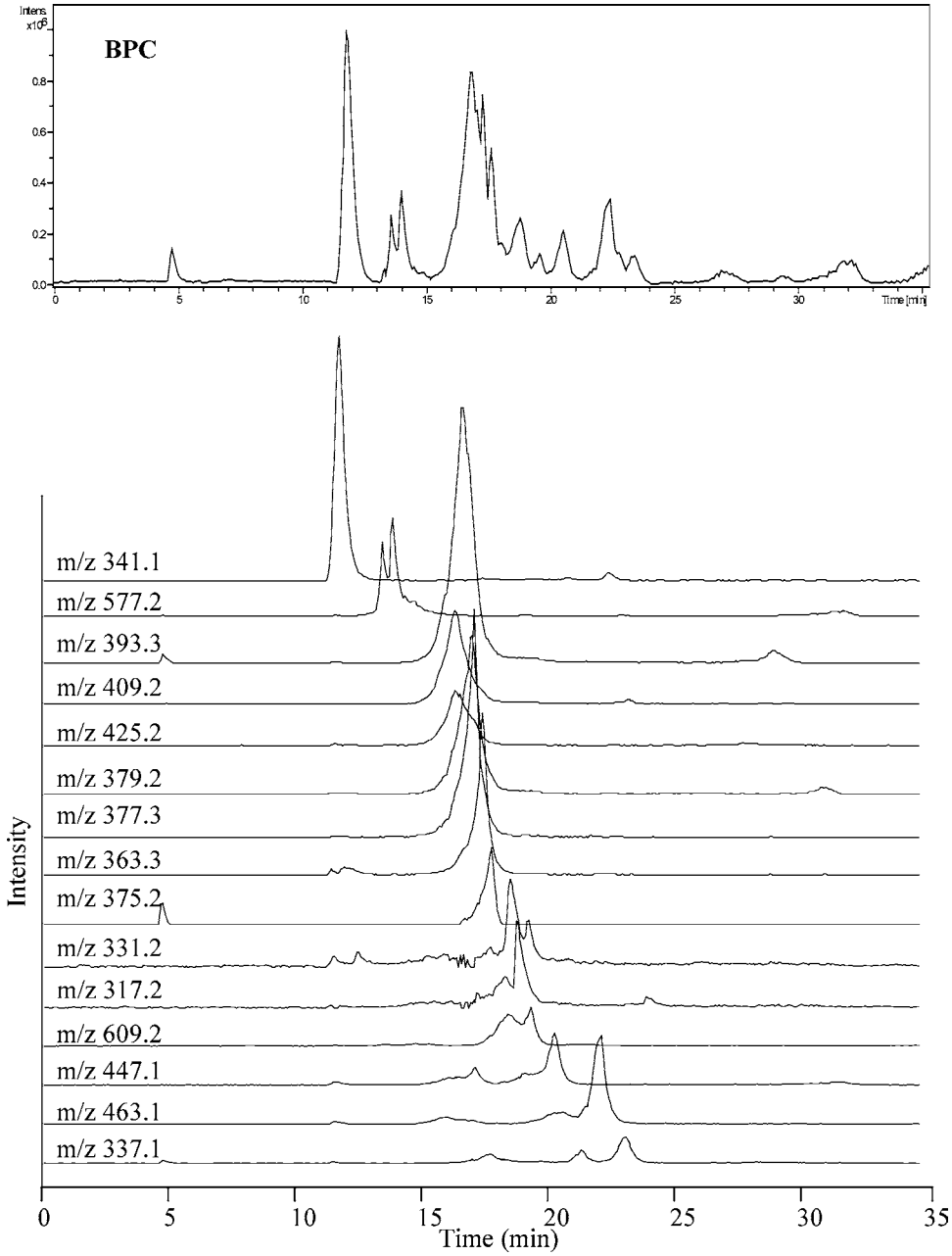

Figure 2. Base peak electropherogram and extracted ion electropherogram. Conditions: buffer ammonium carbonate/ammonium hydroxide, 160 mM at pH 9; 50 μm i.d. fused-silica capillary, 100 cm detector and total length, 20 kV, 7 s of hydrodynamic injection at 0.5 psi; sheath liquid, 2-propanol/water, 50:50 v/v, containing 0.1% TEA; flow rate, 3 μL/min; dry gas, 4 L/min, 150 °C; nebulizing gas pressure, 6 psi. MS analyses were carried out using negative polarity. Compound stability was 25%.

The pH and concentration using ammonium hydroxide/acetic acid as running buffer were also examined. The effect of pH was studied by using a concentration of 500 mM of ammonium hydroxide and adjusting the pH with acetic acid to between 9 and 10. We chose pH 9.5 as the optimum value, and the concentration was then assayed between 100 and 500 mM at this pH. Higher concentrations were tried, but these produced noises in the baseline.

The pH of ammonium acetate/ammonium hydroxide was assayed by varying it between 8 and 11 when using a concentration of 160 mM. The greatest number of peaks appeared at pH 10.5. We then studied the concentration effect between 80 and 180 mM and found the best result with a concentration of 160 mM. **Figure 1** shows the optimum electropherograms found in the different studies carried out with the four running buffers under optimum conditions. Of all of the conditions studied, ammonium carbonate/ammonium hydroxide at pH 9 at a concentration of 160 mM offered the most information about the compounds of interest.

To obtain better resolution between peaks, different percentages (5, 10, and 15%) of organic solvents such as 2-propanol and sodium dodecyl sulfate (SDS) at 5 and 10 mM were added to the buffer without success.

The voltage was varied between 10 and 30 kV, and finally a voltage of 20 kV was selected to obtain the best resolution. The injections were made at the anodic end using N_2 pressure of 0.5 psi for 7 s (1 psi = 6894.76 Pa). These conditions were chosen for the optimization of the ESI parameters.

It is well-known that the choice of sheath liquid has significant effects on sensitivity and on the electrical contact between CE and ESI (*24*, *25*). Generally, a small amount of volatile TEA is used for ESI-negative detection (*26*). Next, the sheath liquid

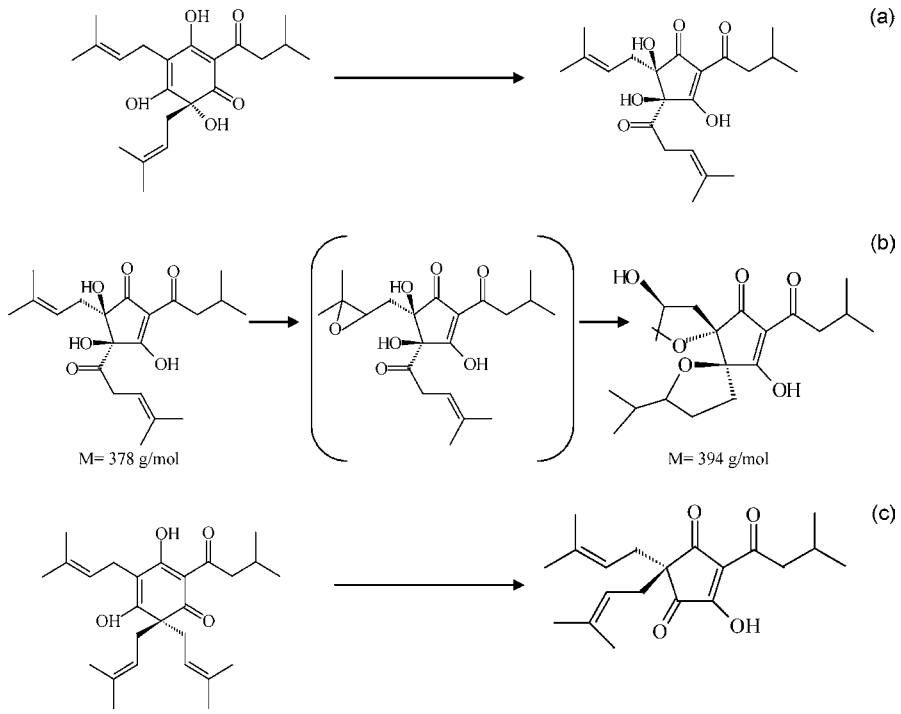

Figure 3. (**a**) Conversion of humulone into humulinone; (**b**) oxidation mechanism for the formation of the compound with molecular weight (M) 394 from humulinone; (**c**) conversion of lupulone into hulupone.

Table 1. Structures of Compounds Found in Different Varieties of Hops without Oxidation

	[M – H][a]			
analyte	Saaz	Nugget	Magnum	Columbus
posthumulone				303.5
cohulupone	317.2		317.2	317.2
hulupone/adhulupone	331.2		331.2	331.2
cohumulone	347.3	347.3	347.3	347.3
humulone/adhumulone	361.2	361.2	361.2	361.3
cohumulinone		363.2		363.2
prehumulone	375.2		375.2	
humulinone/adhumulinone	377.3	377.3	377.3	377.3
colupulone	399.3	399.3	399.3	399.3
lupulone/adlupulone	413.3	413.3	413.3	413.3

[a] [M – H] is the deprotonated ion.

Table 2. Structures of Compounds Found in Different Varieties of Hops with Forced Oxidation

	[M – H][a]			
analyte	Saaz	Nugget	Magnum	Columbus
posthumulone				
cohulupone	317.2	317.2	317.2	317.2
hulupone/adhulupone	331.2	331.2	331.2	331.2
cohumulone		347.3	347.3	347.3
humulone/adhumulone		361.2	361.2	361.2
cohumulinone	363.2	363.2	363.2	363.2
prehumulone	375.2		375.2	
humulinone/adhumulinone	377.3	377.3	377.3	377.3
colupulone		399.3	399.3	
lupulone/adlupulone		413.2	413.2	413.2

[a] [M – H] is the deprotonated ion.

composition and flow rate were optimized to increase the MS sensitivity of the compounds. Different sheath liquids were tested, that is, methanol/water and 2-propanol/water at different proportions, with 0.1% TEA and without TEA. With 2-propanol as organic solvent we obtained a better response than with methanol. Eight different percentages of sheath flow liquid were tested: 2-propanol/water at 40:60 v/v, 50:50 v/v, 60:40 v/v, and 70:30 v/v, with and without 0.1% v/v TEA in order to facilitate electrical contact when the negative mode was used. A 50:50 ratio of propanol/water with 0.1% v/v TEA as sheath liquid was judged to obtain the highest signal and best current stability. The choice of these variables represented a compromise between maintaining efficient electrophoretic separation and improving ionization performance. The influence of the sheath liquid flow rates of 1, 2, 3, 4, and 5 μL/min was also examined. We observed that the best results in terms of MS sensitivity were obtained when using a flow rate of 3 μL/min. The nebulizer pressure was then optimized by testing values of 2, 4, 6, 8, and 10 psi, the greatest sensitivity being obtained with 6 psi. The temperature of the interface was also optimized between 100 and 300 °C, the greatest sensitivity being obtained at 150 °C.

Table 3. Structures of Compounds Found in Naturally Oxidized Saaz and Nugget Hops

	[M – H][a]	
analyte	Saaz	Nugget
posthumulone		
cohulupone	317.2	317.2
hulupone/adhulupone	331.2	331.2
cohumulone	347.3	347.3
humulone/adhumulone		
cohumulinone	363.2	363.2
prehumulone		
humulinone/adhumulinone	377.3	377.3
colupulone		
lupulone/adlupulone		

[a] [M – H] is the deprotonated ion.

Another important parameter of the interface was the stability of the compound, which was studied between 25 and 100%. The MS signal decreased concomitantly with higher percentages because the number of molecules transferred into MS was low, whereas with lower percentages the majority of the compounds

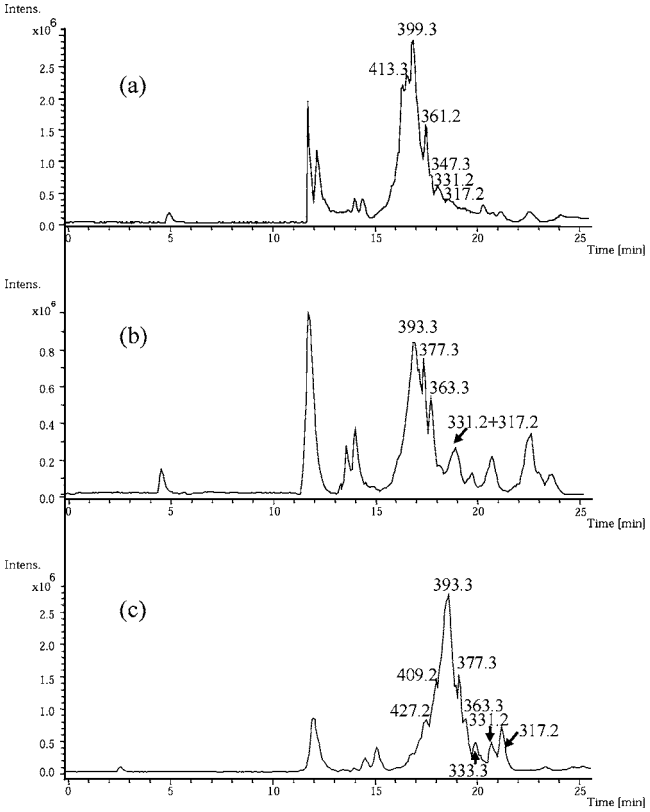

Figure 4. Differences among samples of Saaz hops: (**a**) without oxidation; (**b**) with forced oxidation; (**c**) naturally oxidized. The separation conditions are shown in **Figure 2**.

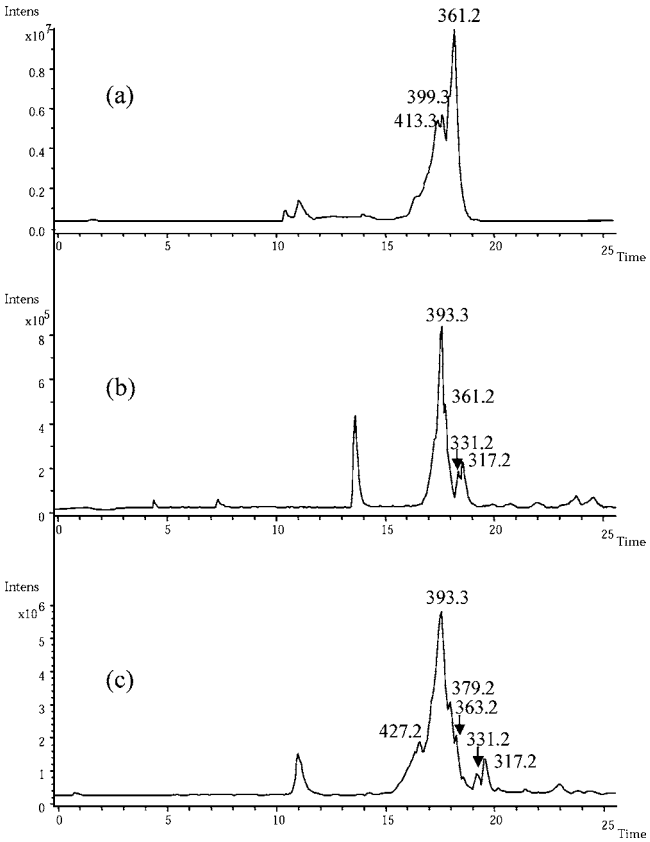

Figure 5. Differences among samples of Nugget hops: (**a**) without oxidation; (**b**) with forced oxidation; (**c**) naturally oxidized. The separation conditions are shown in **Figure 2**.

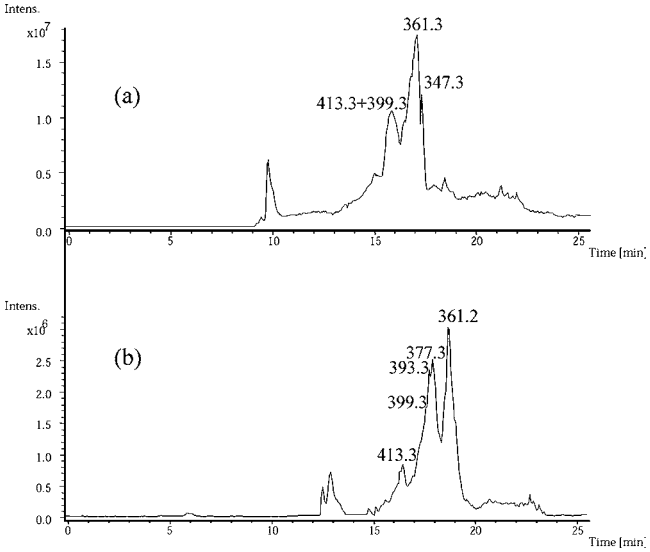

Figure 6. Differences among samples of Magnum hops: (**a**) without oxidation; (**b**) with forced oxidation. The separation conditions are shown in **Figure 2**.

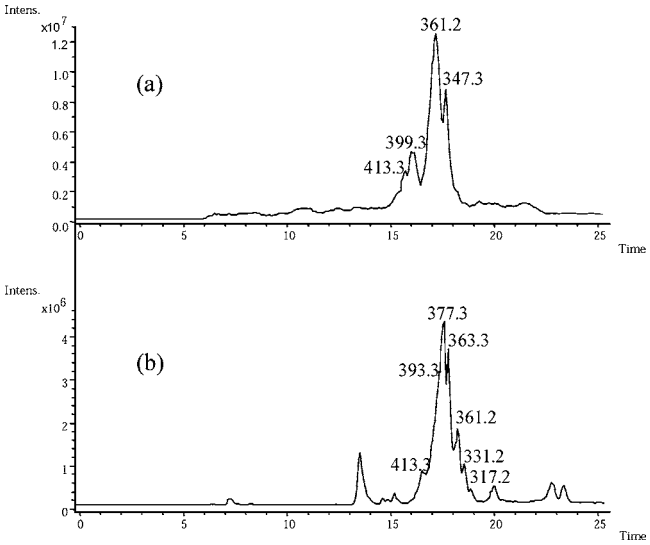

Figure 7. Differences among samples of Columbus hops: (**a**) without oxidation; (**b**) with forced oxidation. The separation conditions are shown in **Figure 2**.

became more stable, as indicated by an increase in the MS signal. This parameter is related to the voltage used in the capillary placed at the MS entrance; thus, the higher this parameter, the higher the voltage applied by the MS instrument and therefore the higher the solute fragmentation that can take place at that point. Thus, we chose 25% compound stability.

Figure 2 shows the base peak electropherogram and the extracted ion electropherogram obtained under the CE-ESI-MS conditions chosen for an extract of oxidized Saaz hops. Fifteen different compounds can be recognized: *m*/*z* 377.3 corresponds to humulinone/adhumulinone (overlap), oxidation products of humulone/adhumulone. Oxidation occurs leading to the creation of a double acyloin entity. The acyloin rearrangement with concurrent ring contraction may take place at both C-4 and C-6. This reaction is totally analogous to the important isomerization reaction of humulone to the isohumulones (**Figure 3a**); an ion with a ratio *m*/*z* of 393.3 indicates that two oxygen atoms have been incorporated into humulone/adhumulone (overlap). This

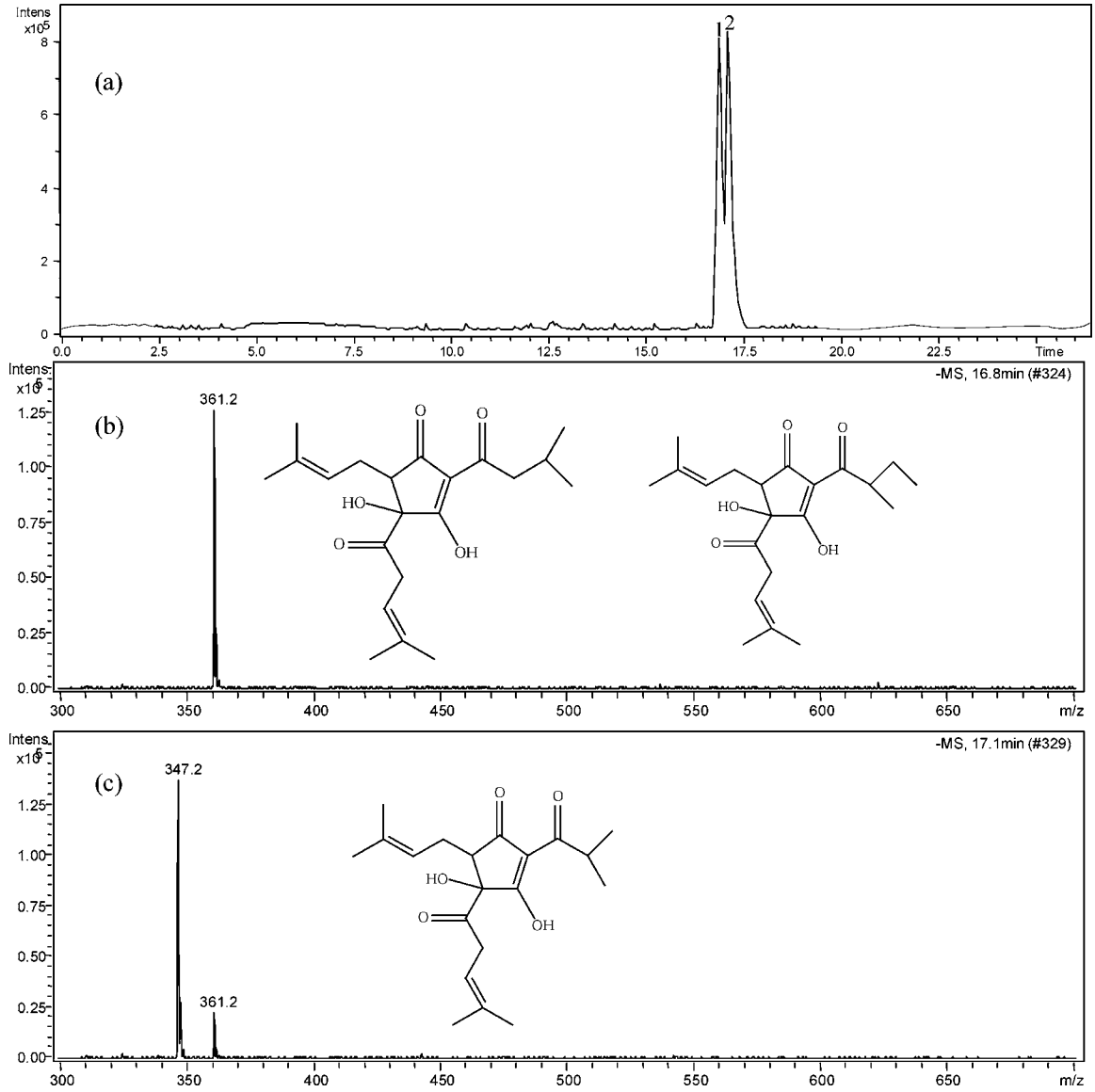

Figure 8. (**a**) Electropherogram of a mixture of three trans iso-α-acid standards: 1, *trans*-iso-humulone and *trans*-iso-adhumulone; 2, iso-cohumulone. (**b**) Mass spectra of isohumulone and iso-adhumulone with a *m*/*z* ratio of 361.2. (**c**) Mass spectra of iso-cohumulone with a *m*/*z* ratio of 347.2.

oxidation product belongs to the abeo-isohumulone group, which is derived from isohumulones but obtained directly through the oxidation of humulone. It is very likely that humulinone represents the first step in the reaction sequence. These oxidized compounds have a five-membered structure. The reaction mechanism of these compounds (**Figure 3b**) proceeds via the oxidation of the 3-methyl-2-butenyl side chains in humulinone, followed by cyclization, via either intramolecular dehydration or nucleophilic cleavage of the intermediate oxirane ring (*1*). The ions with *m*/*z* ratios of 409.2 and 425.2 are thought to be more highly oxidized compounds, thus indicating that three and four oxygen atoms, respectively, have been incorporated into humulone/adhumulone, but their structure and the formation mechanism are still unknown; *m*/*z* 363.3 corresponds to cohumulinone, an oxidation product of cohumulone, with a structure and oxidation mechanism similar to that of humulinone, and the *m*/*z* of 379.2 could be due to the incorporation of two oxygen atoms into cohumulone, in the same way as with humulone; *m*/*z* 331.2, hulupone/adhulupone (overlap), and *m*/*z* 317.2, cohulupone, correspond to the oxidation of the β-acids lupulone/adlupulone and colupulone. The structures of the oxidation products indicate that the lengths of the side chains in these compounds, together with the double bonds and hydroxyl groups, easily give rise to oxidation cyclizations, leading to five derivatives (**Figure 3c**) (*m*/*z* 375.2, prehumulone). Other compounds have been found in this extract: *m*/*z* 341.1, maltose; *m*/*z* 577.2, procyanidin; *m*/*z* 609.2, hesperedin (18.6 min) and rutin (19.5 min); *m*/*z* 447.1, luteolin-7-*O*-glucoside (17.2 min) and kaempferol-3-*O*-glucoside (20.5 min); *m*/*z* 463.1, quercetin-4′-*O*-glucoside; and *m*/*z* 337.1, desmethylxanthohumol. Some of them have easily been identified and confirmed using the hop acid standard.

The reproducibility of the CE-ESI-MS analysis, expressed by the relative standard deviation (RSD) of six consecutive injections, was 3.6% for the retention time and 6.9% for the peak area, both quite suitable for the intentions of this work.

Analysis of Acids in Different Hop Varieties. To demonstrate the capacity of the CE-ESI-MS method for the analysis of this type of compound in hop samples, we applied the method to different varieties. Four varieties were studied: Saaz (a classic variety with good aroma but poor storage stability); Nugget and Magnum (with similar properties of high α-acid, acceptable aroma, and good storage stability); and Columbus (with high α-acid, a strong but pleasant aroma, but poor storage stability).

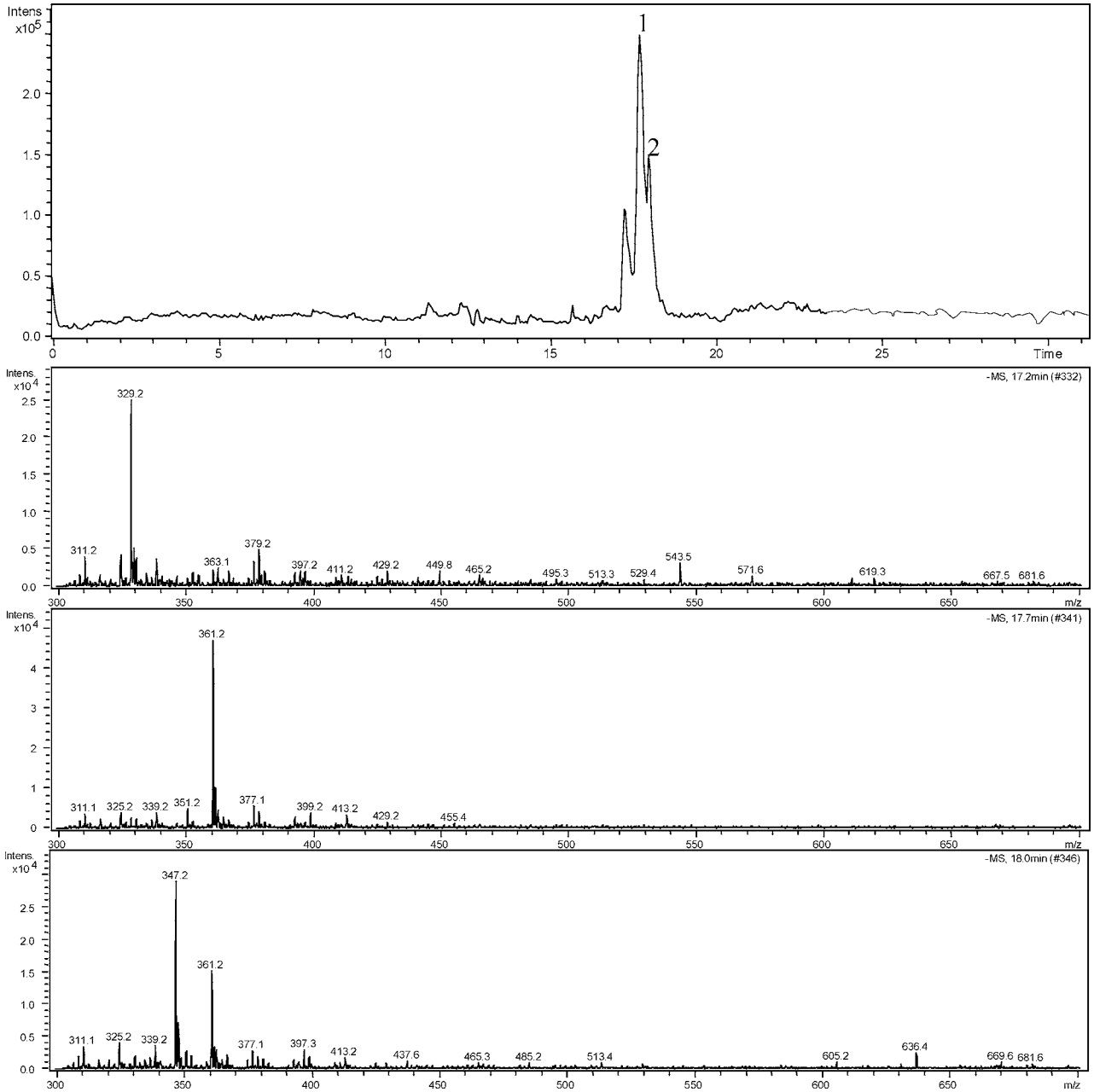

Figure 9. Electropherogram and mass spectra of an "extra" beer. The separation conditions are shown in **Figure 2** and the peak numbers in **Figure 8**.

Table 1 shows information about the structures of the compounds found in the four varieties of hop with no oxidation. As can be seen in this table, *m*/*z* 377.3, which corresponds to humulinone/adhumulinone, appears in all varieties. Humulinone is perhaps the best known nonvolatile oxidation product derived from humulone. Nugget hop is the variety with the best result because it contains fewer oxidized compounds, only cohumulinone (*m*/*z* 363.2), humulinone/adhumulinone (*m*/*z* 377.3), and another oxidation product of humulone with *m*/*z* 409.3. Columbus is the variety with the poorest storage stability, and so it contains more oxidized compounds; in addition to some of the components present in Nugget we also found cohulupone (*m*/*z* 317.2) and hulupone/adhulupone (*m*/*z* 331.2). These oxidation derivatives also appear in the varieties Saaz and Magnum, although the oxidation compound of cohumulone (*m*/*z* 363.2) does not appear in these varieties.

Analysis of Oxidation Derivatives in Different Varieties of Hops. The oxidation of hops was forced by heating 10 g to 80 °C for 2 h. We also studied two varieties, Nugget and Saaz, oxidized at room temperature for about 2 years. **Table 2** shows information about the structures of the compounds found in the four varieties of hops subjected to forced oxidation. The presence of characteristic hop compounds such as humulone/adhumulone (*m*/*z* 361.2) can be seen in the varieties Nugget, Magnum, and Columbus together with cohumulone (*m*/*z* 347.3) and lupulone/adlupulone (*m*/*z* 413.2). Colupulone (*m*/*z* 399.3) is to be found in only Nugget and Magnum. Apart from this, the typical oxidized compounds of α-acids [humulinone/adhumulinone (*m*/*z* 377.3) and cohumulinone (*m*/*z* 363.2)] and *β*-acids [hulupone/adhulupone (*m*/*z* 331.2) and cohulupone (*m*/*z* 317.2)] were to be found in all of the varieties. It can also be seen that the majority of α- and *β*-acids disappeared when we forced the oxidation in Saaz. Colupulone disappeared in Columbus. Other oxidation products have been found (*m*/*z* 379.2, 393.3, 409.2, 425.2, and 427.2), but they are not shown in the table.

For naturally oxidized Saaz and Nugget hops, only oxidation compounds appear, except for cohumulone, due to oxidation being more extensive over a longer period of time (**Table 3**).

Figure 4 shows the differences between samples of Saaz hops. We found that in the Saaz hops subjected to both forced and natural oxidation the maximum peak was at 393.3, corresponding to the gain of two oxygens into humulone. **Figure 5** shows the differences among hop Nugget without oxidation, hop Nugget with forced oxidation, and naturally oxidized hop Nugget. The same applied to the Nugget variety where both oxidized forms showed a maximum peak at 393.3.

Figure 6 shows the electropherogram of the samples of hop Magnum: hop Magnum without oxidation and hop Magnum with forced oxidation. With the Magnum variety we can detect the presence of peaks with *m*/*z* ratios of 361.2, corresponding to humulone/adhumulone, 399.3, corresponding to colupulone, and 413.3, corresponding to lupulone/adlupulone in both oxidized and nonoxidized hops.

Figure 7 shows the electropherogram of the samples of hop Columbus: hop Columbus without oxidation and hop Columbus with forced oxidation. In this variety, the most characteristic oxidation compounds found in Columbus are humulinone/adhumulinone (*m*/*z* 377.3) and cohumulinone (*m*/*z* 363.3).

Comparing the results for the four samples, we can deduce that the Saaz variety contains fewer hop acids than the other varieties. Nugget samples with no oxidation show the least number of oxidized derivatives, and thus this variety has the best storage stability. Magnum has the highest α- and *β*-acid contents.

Determination of Iso-α-acids in "Extra" Beer. Iso-α-acids, hop-derived compounds present in low concentrations in beers, are primary flavor constituents. The bitterness of beer is largely attributable to iso-α-acids, which are formed by isomerization during the wort-boiling stage of beer production (*3*).

The identification and quantification of iso-α-acids in an "extra" beer was carried out using the CE-ESI-MS method proposed, after preconcentrating 100 mL of the beer sample using a DSC-C18 column. The sample was recovered by passing it through four portions of 5 mL of acetone/water (75:25 v/v). The extract was dried, reconstituted in 2 mL of acetone/water (50:50 v/v), and filtered before analysis. The recovery percentage was ≈70% for the iso-α-acids using this extraction system. **Figure 8a** shows the electropherogram of a mixture of three iso-α-acid standards, **Figure 8b** the mass spectra of isohumulone and iso-adhumulone, which have an *m*/*z* ratio of 361.2, and **Figure 8c** the mass spectra of iso-cohumulone, which has an *m*/*z* ratio of 347.2. **Figure 9** shows the electropherogram and the mass spectra of an "extra" beer. As can be seen in this figure an unknown peak appears at *m*/*z* 329.2, and the iso-α-acids appear at *m*/*z* 361.2 and 347.2.

The quantification was carried out using calibration graphs studied between 25 and 500 mg L^{-1}. The correlation coefficients (r^2) obtained for the regression lines of the CZE plots of peak area versus concentration were all >0.998.

Detection limits for the analytes were 2.00 mg L^{-1} for isohumulone + iso-adhumulone and 2.22 mg L^{-1} for iso-cohumulone, and the limits of quantification were 6.67 mg L^{-1} for iso-humulone + iso-adhumulone and 7.41 mg L^{-1} for iso-cohumulone, all of which were calculated by the IUPAC (*27*).

The precision of the method was evaluated by determining the repeatability of the peak areas. The repeatability values obtained for three successive injections of a standard solution of 300 mg L^{-1} for each analyte were ≈5.7%.

The concentration found for isohumulone + iso-adhumulone was 2.26 mg L^{-1} and that for iso-cohumulone, 1.05 mg L^{-1}, in the initial beer sample, which was with Saaz hops. This variety is characterized by its high aroma and its low α-acid content, which in turn implies a low iso-α-acid content.

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