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Analysis of hop acids by capillary electrophoresis

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Abstract

Micellar electrokinetic chromatography (MEKC) was used to separate components of hop extracts. The separation of a sample of iso-α-acids by MEKC was better and faster than by an established HPLC method, giving $<0.8\%$ RSD on migration times and 5–10% RSD on peak areas. MEKC was also successfully used to separate the oxidation products of the α- and β-acids and thus to monitor the stability of hop products containing them. Furthermore, MEKC distinguished among samples of reduced iso-α-acids (rho-, tetrahydro- and hexahydro- derivatives).

Keywords: Hops; Hop acids; Iso-α-acids; Iso-α-acid derivatives; Capillary electrophoresis; Micellar electrokinetic chromatography

1. Introduction

Although brewing of beer with hops has a long history, until the latter part of the last century it has been more an art than a science. Traditionally, beer was ''hopped'' by adding dried hop cones (female flowers of the species *Humulus lupulus* L.) to the wort kettle during the boiling step of beer making. The addition of hops was found to improve the quality of the beer as well as imparting the characteristic bitter taste, which was eventually to find favour with consumers. This bitter taste is the result of isomerisation of the α-acids, found in the hop cones, during the long boil. In the modern brewing process, it is more common to use products that have been extracted from hops and, increasingly, in forms that can be added post-fermentation, since this allows better control of the bitter taste. These hop products include the α-acids that have been pre-isomerized (iso-α-acids), and also products that have been reduced using hydrogen in the presence of a noble metal catalyst (tetrahydro iso-α-acids) or with borohydride (rho iso-α-acids) or both (hexahydro iso-α-acids; Fig. 1). Each of these products imparts to the beer a different flavour profile and rho-, tetra-, and hexahydro- iso-α-acids have the additional benefit of protecting the beer from ''light struck'', allowing it to be offered for sale in clear and green glass bottles. It is now common practice to use iso-α-acids and their derivatives in the form of mixtures. Thus, the analysis of such mixtures is of importance to the industry.

The traditional method for measuring bitterness in beer, i.e. spectrophotometric analysis, is incapable of distinguishing between the source of bitterness and is therefore wholly unsuitable when any mixture is involved. A better method is high performance liquid chromatography (HPLC), which, under isocratic conditions, can successfully separate iso-α-acids in the presence of α- and β-acids (Wilson, 1989). However, analysis by HPLC becomes difficult and time consuming if other mixtures or very complex mixtures of acids, need to be separated, due to their sensitivity to trace metals in HPLC columns and commercial stationary phases (Verzele & De Keukeleire, 1991; Wilson, 1989). Furthermore, reproducibility problems are sometimes encountered. Thus, reliable methods for the separation of such mixtures, especially of iso-α-acids and their derivatives that are simple to use are of considerable interest.

Capillary electrophoresis (CE), in the micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC) modes has been applied to the analysis of hop acids (De Keukeleire, David, Haghebaert, & Sandra, 1998; Sandra,

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Haghebaert, Szücs, & David, 1996; Szücs, Van Hove, & Sandra, 1996; Szücs, Vindevogel, Everaert, Decooman, Sandra, & De Keukeleire, 1994; Vindevogel & Sandra, 1991; Vindevogel, Sandra &, Verhagen, 1990). The α-acids (*n*-humulone, cohumulone and adhumulone) and β-acids (*n*-lupulone, colupulone and adlupulone) were separated, the best separation being achieved using MEEKC (Szücs et al., 1994).

The aims of this work were to apply, for the first time, MEKC to the separation of iso-α-acids and their reduced derivatives, i.e. the rho-, tetra- and hexa-iso-α-acids, to examine the potential use of MEKC to monitor the stability of an iso-α-acid extract, and to investigate the ability of MEKC to distinguish among samples of reduced iso-α-acids.

2. Experimental

2.1. Samples

Eight samples were analyzed and are described in Table 1. They comprised a hop extract (A), prepared by extracting with carbon dioxide at 6 MPa and 10°C, the four most widely available post fermentation bittering products (B, F, G, H) and three samples prepared specifically to aid peak identification (C, D, E).

2.2. Reagents

Buffers were purchased from Hewlett Packard (Bracknell, UK) as 50 mM borate at pH 9.3 or as 50 mM borate containing 50 mM SDS at pH 9.3. They were mixed 1:4 (v/v) to give 50 mM borate containing 40 mM SDS at pH 9.3, which was filtered through a 0.2 μm membrane before use. Methanol (HPLC grade) was from Rathburn, UK. High purity water was prepared in-house with a Purite Lab-water RO50 unit (Purite Ltd, High Wycombe, UK).

2.3. MEKC

Separations were performed on a Hewlett-Packard (Bracknell, UK) HP^{3D}CE with diode-array detection and an HP^{3D}CE ChemStation for control of the instrument and acquisition and analysis of data. Separations

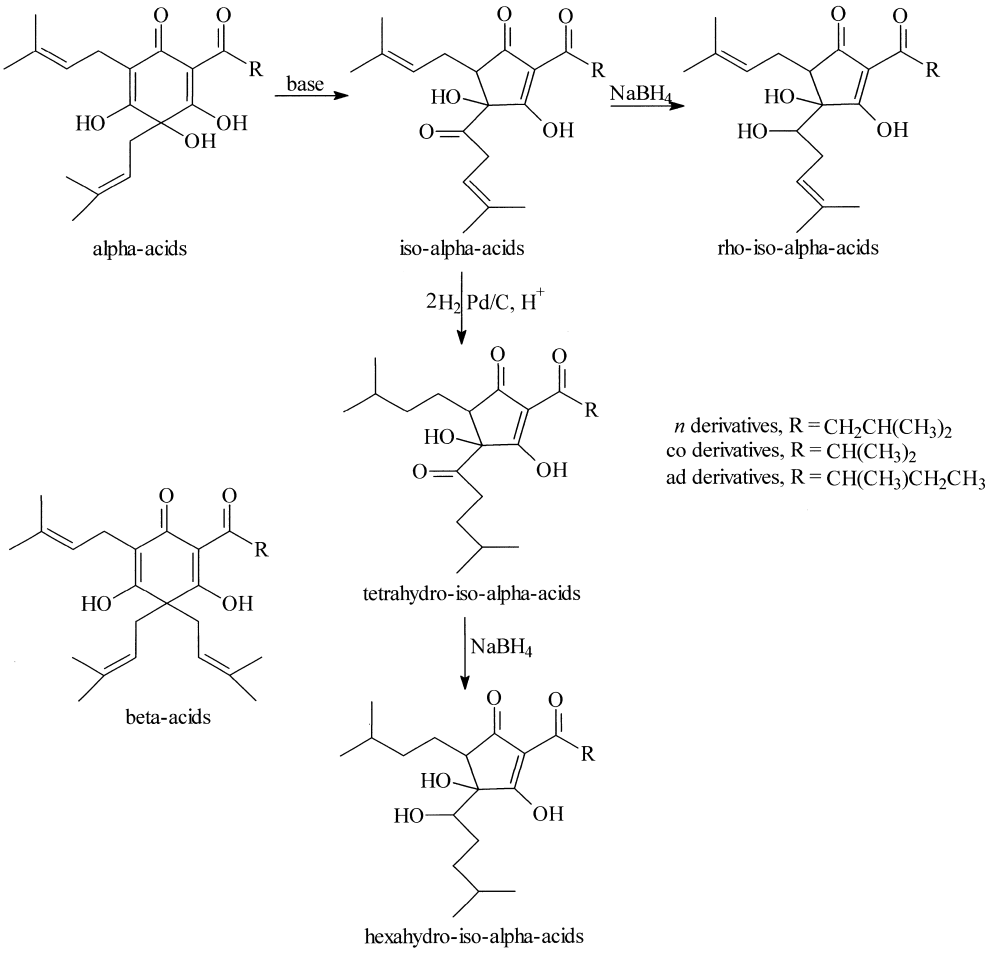

Fig. 1. Structures of the hop acids analyzed.

were performed in a fused silica capillary (64.5 cm total length and 56 cm to the detector, 50 μm i.d.) using 50 mM borate buffer containing 40 mM SDS at pH 9.3. Samples were introduced hydrodynamically (50 mBar for 5 s) and then run at 25 kV. Separations were monitored at 200 nm, with full spectral collection from 190–600 nm. Prior to each run, the capillary was flushed for 3 min with either 0.1 M NaOH or methanol followed by 3 min with buffer. All samples were filtered through a 0.2 μm filter, prior to analysis.

3. Results and discussion

Initial MEKC method development involved running samples A and B in borate buffer with a range of SDS concentrations. At 40 mM SDS, good resolution of components was achieved in a reasonably short run time. The capillary was flushed at 1 Bar with 0.1 M NaOH followed by buffer, each for 3 min, prior to each run.

3.1. Liquid carbon dioxide hop extract (sample A)

Fig. 2 shows the e-gram for sample A prepared in methanol and the order of migration confirms that obtained by Szücs et al. (1996). Identification of components was based on their diode array spectra, the humulones giving different spectra from the lupulones (Fig. 3) and on the migration times and ratios of components of samples C, D and E. Peak area/migration time values gave a measure of relative amounts of each component in the different samples and the percentages of the α- and β-acids in samples A, C, D and E are given in Table 2.

Table 1
Description of samples analyzed

Sample code	Description
A	Carbon dioxide extract of the variety Target containing 52.1% (w/w) α-acids and 20.3% (w/w) β-acids with the remainder made up of minor soft resin components, volatile oil, fat, wax and water.
B	Iso-α-acid solution containing 30% (w/v) iso-α-acids in their potassium salt forms in water.
C	Concentrated α-acid sample containing 73.3% (w/w) α-acids (30.3 cohumulone 30.3% and 43.3% *n*+adhumulone) made by carrying out a primary alkali extraction of a liquid carbon dioxide extract of hops which was subsequently acidified.
D	Concentrated β-acid sample containing 65.0% (w/w) β-acids (38.2% colupulone and 26.8% *n*+adlupulone) made by carrying out a secondary alkali extraction of a liquid carbon dioxide extract of hops which was subsequently acidified.
E	Concentrated colupulone sample containing 79.6% (w/w) β-acids (66.2% colupulone and 13.4% *n*+adlupulone) made by carrying out an alkali extraction of a concentrated β-acid sample which was subsequently acidified.
F	Rho iso-α-acid solution containing 35% (w/v) rho iso-α-acids in their potassium salt forms in water made by carrying out a borohydride reduction of iso-α-acids.
G	Tetrahydro iso-α-acid solution containing 10% (w/v) tetrahydro iso-α-acids in their potassium salt forms in water.
H	Hexahydro iso-α-acid solution containing 20% hexahydro iso-α-acids in their potassium salt forms in water. Made by carrying out a borohydride reduction of tetrahydro iso-α-acids.

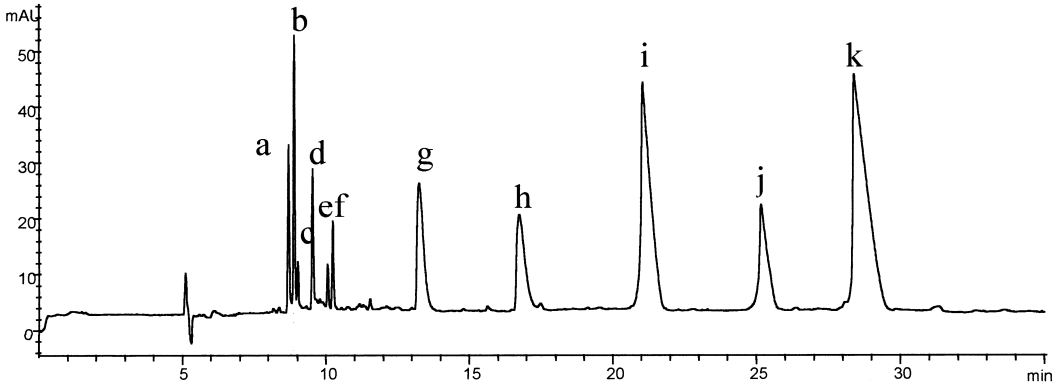

Fig. 2. E-grams (200 nm) of sample A. Peaks a–f, probable oxidation products of α- and β-acids; g, colupulone; h, *n*- and/or adlupulone; i, cohumulone; j, adhumulone; k, *n*-humulone.

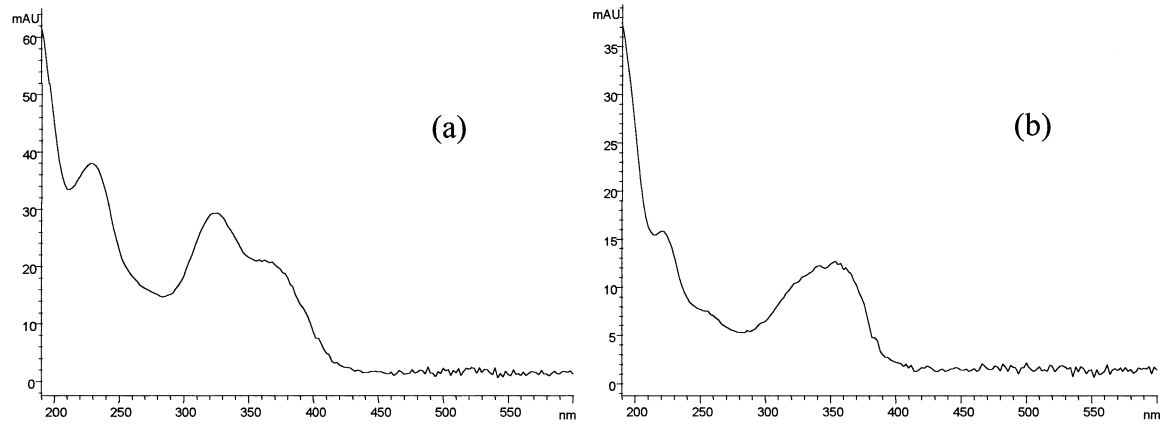

Fig. 3. Diode array spectra for (a) humulones and (b) lupulones.

A series of six small peaks (a–f) was separated from sample A, migrating between 8.5 and 10.5 min when the sample was prepared in methanol (Fig. 2). It is well known by the industry that the α- and β-acids are not stable in methanol. Peaks a–f are likely to be oxidation products of the humulones and lupulones since they possess diode array spectra which were similar to those observed for the lupulones of sample A (Fig. 3b). Analysis of samples C and D (concentrated α-acids and β-acids, respectively) showed that peaks a, c and d were oxidation products of humulones while peaks b, e and f were oxidation products of lupulones. For sample A, it was observed that the peak areas of the humulones and lupulones decreased while those of the oxidation products increased with time (0–6 h) between sample preparation and analysis (Fig. 4), indicating the importance of analysing samples as soon as possible after preparation.

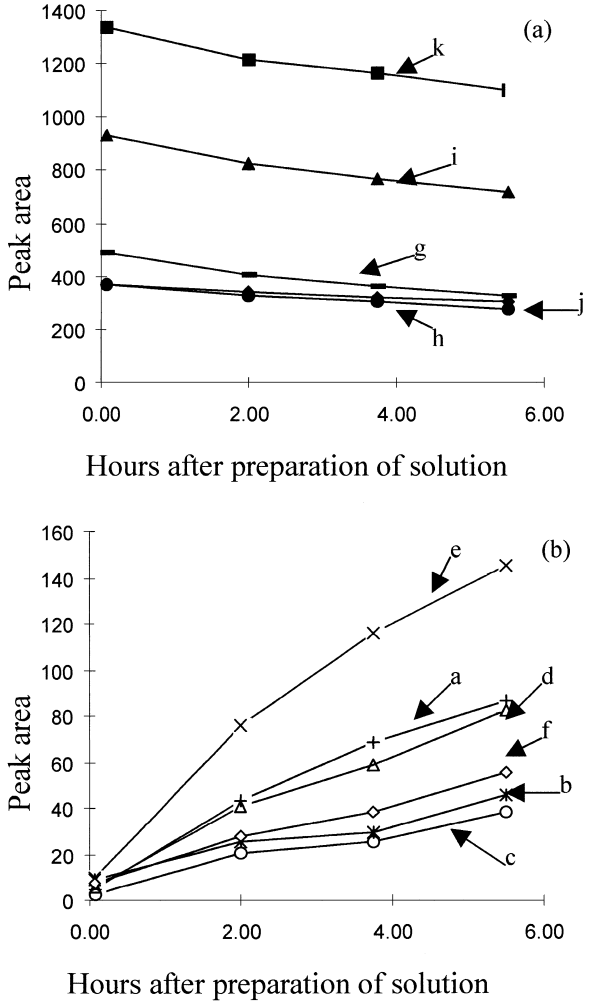

Fig. 4. Change in peak areas of (mAU*s) (a) humulones and lupulones and (b) their oxidation products with time between preparation and analysis of sample A.

3.2. Iso-α-acids (sample B)

Nine peaks (1–9) were obtained which all possessed different migration times to those observed for the α- and β-acids and their degradation products separated from sample A. Expanded e-grams (over the range 7.5

Table 2
Percentage composition of the humulone and lupulone fractions of samples A, C, D and E

	Experimental[a] (specified[b]) data			
Acid	A	C	D	E
Colupulone	18.57 (nd[d])	na[c]	56.79 (58.8)	79.43 (83.2)
n- and Adlupulone	13.08 (nd)	na	43.32 (41.2)	20.58 (16.8)
Total lupulones	31.63 (28.0)	na	na	na
Cohumulone	28.23 (nd)	44.44 (41.3)	na	na
Adhumulone	9.74 (nd)	11.16 (nd)	na	na
n-Humulone	30.40 (nd)	44.40 (nd)	na	na
n-+Adhumulone	40.14 (nd)	55.56 (59.1)	na	na
Total humulones	68.37 (72.0)	na	na	na

[a] Percentages calculated using peak area/migration time data collected at 200 nm for each α- and/or β-acid. Quoted values are the average of duplicate determinations.

[b] Quoted values are the percentage composition of each acid as a proportion of the total α- and/or β-acids in the sample, as specified in Table 1.

[c] nd, not declared.

to 13 min) for samples A and B are shown in Fig. 5(a), (b). The spectra of the iso-α-acids were also different from those of the α- and β-acids [Fig. 3(a), (b)]; peaks 1, 2, 3, 5, 8 and 9 having spectra of the type shown in Fig. 5(c), while the spectrum shown in Fig. 5(d) was typical of peaks 4, 6 and 7. Separation of the iso-α-acids by MEKC was much better than that reported by HPLC (Wilson, 1989). The number of peaks obtained by MEKC and also their spectra being different from those of the α- and β-acids suggested the separation of soft resin components with structures related to those of the isohumulones.

Due to problems with samples coating the capillary wall, the analytical method was improved by modifying the conditioning protocol, i.e., 3 min with methanol (instead of 0.1 M NaOH) followed by 3 min buffer, flushed at 1 Bar. This reduced the total run time for sample A from 30 min to 20 min and for sample B from 13 min to 10 min. This improved method was adopted for the re-analysis of sample B and to analyze the reduced iso-α-acids (samples F–H) described below.

The 200 nm e-grams for the iso-α-acids (sample B), obtained using the modified capillary conditioning procedures are shown in Fig. 6(a). Ten repeat injections of sample B on a capillary conditioned between runs with methanol and buffer gave repeatabilities of $<0.8\%$ RSD on migration times. The RSDs on peak areas were between 5 and 10% but this included a gradual fall in peak area over time since each run was started ca. 22 min after the previous one. As these samples are

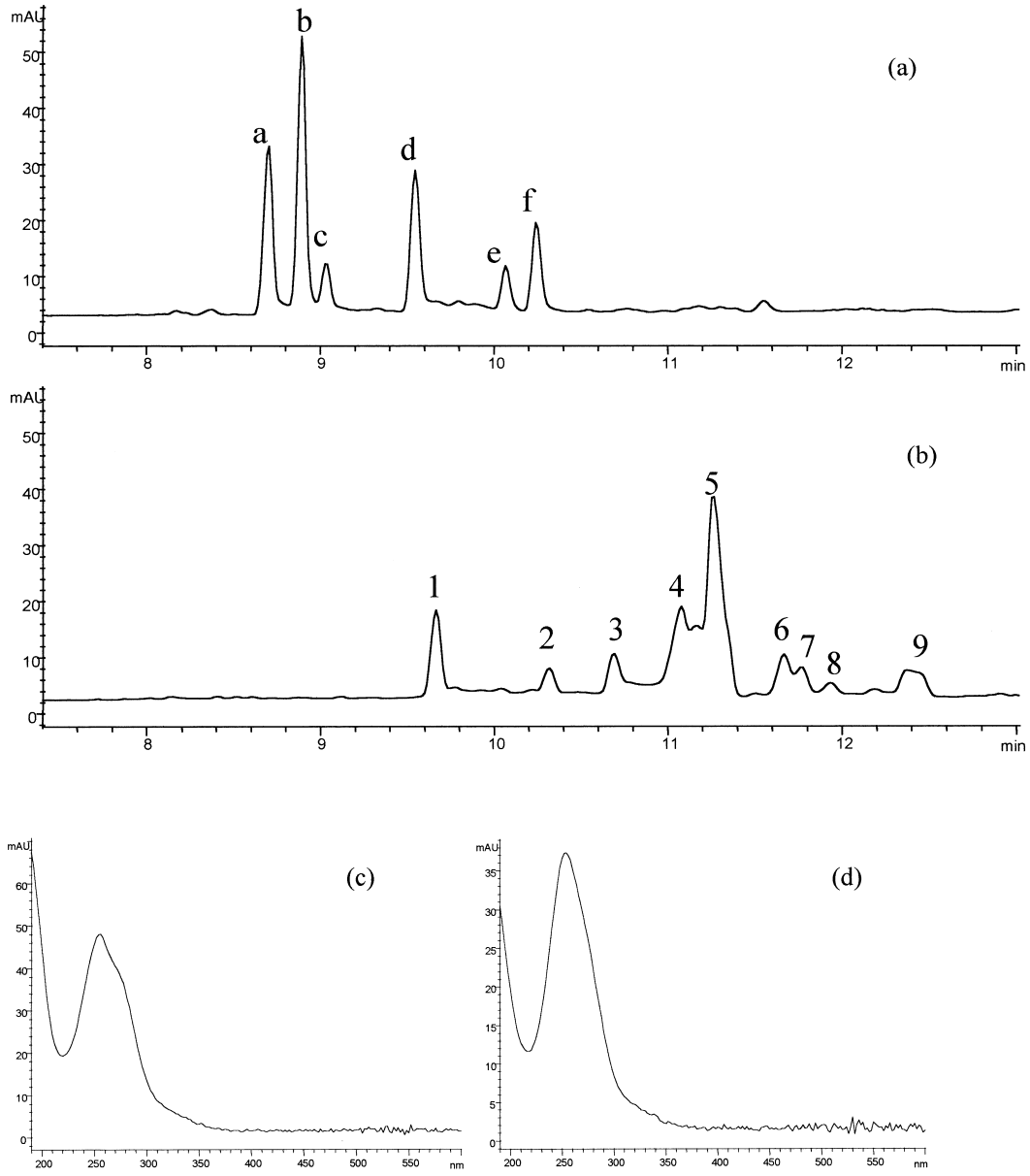

Fig. 5. Expanded 200 nm e-grams for (a) sample A and (b) sample B and typical diode array spectra for (c) peaks 1, 2, 3, 5, 8 and 9 and (d) peaks 4, 6 and 7.

known by the industry not to be fully stable in methanol, the real repeatability values on peak areas would be higher than those quoted here. Preparation of samples in ethanol, rather than methanol, should greatly enhance their stability.

3.3. Reduced iso-α-acids (samples F–H)

All the reduced iso-α-acids were analyzed using the improved MEKC method, incorporating the modified conditioning protocol. The rho-iso-α-acids (sample F) gave one main peak migrating at 8.35 min which possessed a shoulder (migration time 8.51 min), as shown in Figs. 6(b) and representing the major components of the sample. More work, using different buffer compositions, is required to improve the separation of this sample. Nevertheless, MEKC using the current improved method clearly shows differences between the iso-α-acids and the rho-iso-α-acids.

Samples G and H gave three main peaks for each sample [Fig. 6(c), (d)] and, in addition, three smaller peaks (sample G) or a shoulder on each main peak (sample H), suggesting the separation, or partial separation, of the *cis* and *trans* isomers.

The UV spectra of the iso-α-acids and their reduced products were indistinguishable from each other but they were easily distinguished from the α- and β-acids [Fig. 5(a–c)]. Therefore, the migration times of the reduced acids relative to each other are the primary means of identification.

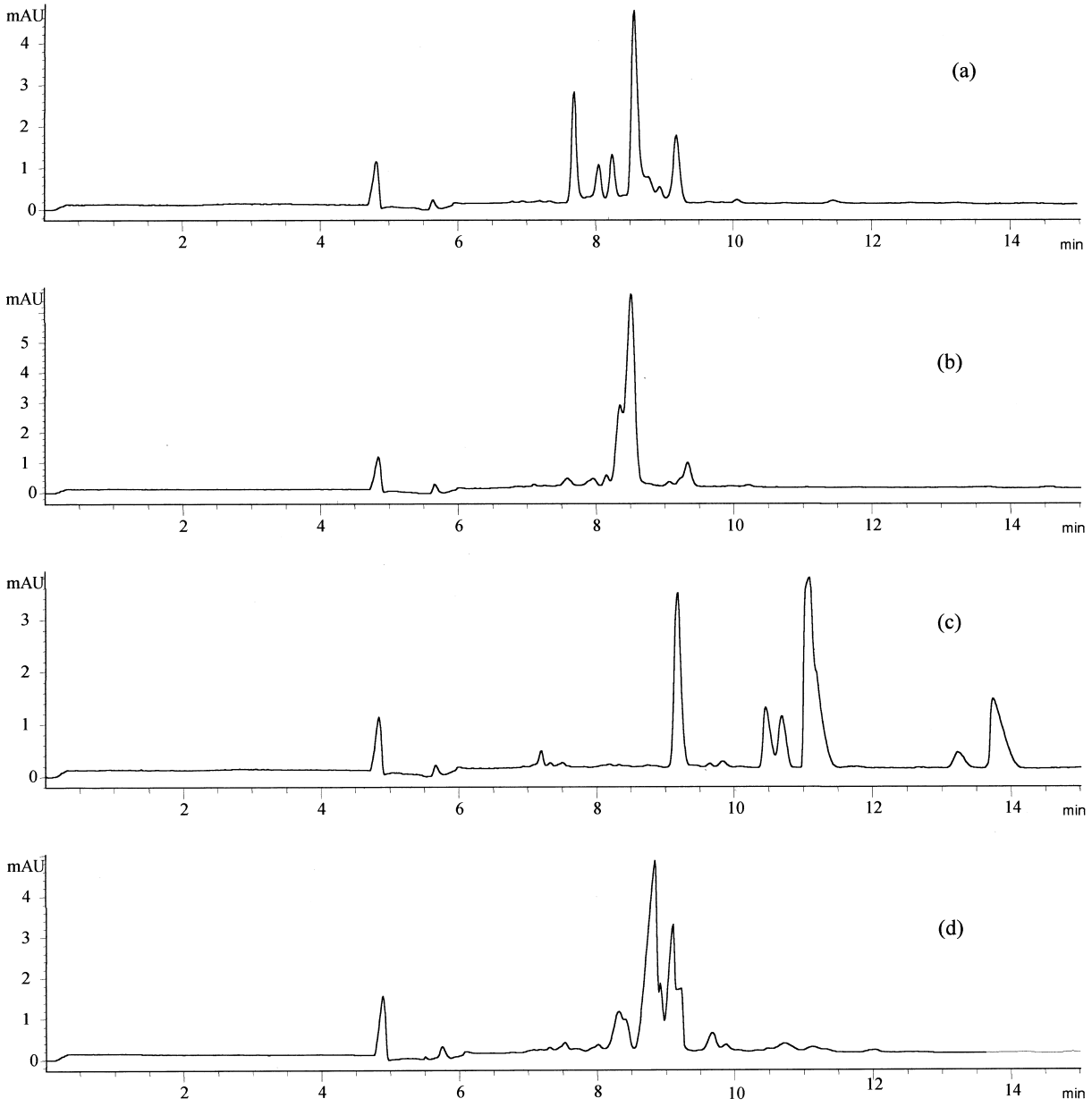

Fig. 6. E-grams (200 nm) for (a) sample B, (b) sample F, (c) sample G and (d) sample H, using the modified MEKC conditions (see text).

4. Conclusion

There are several new findings from this study. MEKC gives very reproducible separation of hop iso-α-acids and the separation is better than that reported by HPLC (Wilson, 1989). MEKC can be used to separate the oxidation products of the α- and β-acids and thus monitor the stability of hop products containing them. MEKC can readily distinguish among reduced iso-α-acids, i.e. the rho-, tetra- and hexa- derivatives.

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