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Note

Separation of α-, β- and iso-α-acids in hop products and beer by high-performance liquid chromatography

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The analysis of hop iso-α-acids has usually been carried out using a procedure involving isooctane extraction and direct photometry of the extract[1]. Recently a method was reported allowing the iso-α-acid to be measured, after evaporation of the extract, by high-performance liquid chromatography (HPLC)[2], followed by a method of iso-α-acid measurement by HPLC directly from the beer sample[3]. Methods of HPLC measurement of α-acid and β-acid have also been reported[4].

Detailed here is an isocratic method of separation of α-acid, β-acid and iso-α-acid involving at maximum 13 min per run to analyse β-acid content. As little sample pretreatment is required, the method may be used for the rapid measurement of these specific hop substances in beer.

EXPERIMENTAL

Chromatography was carried out using a 6000A solvent pump (Waters, Hartford, Great Britain) and an Altex (Beckman RIIC, High Wycombe, Great Britain) 112 injection valve with a 20-μl sample loop, together with a Cecil CE212 variable-wavelength spectrophotometer (Cecil Instruments, Cambridge, Great Britain) with a flow-through cell measuring at 270 nm. The chromatograms were plotted on a Servoscribe RE541 potentiometric recorder and integrated on a Hewlett-Packard 3390A integrator. The column was 25 cm × 4.6 mm I.D. stainless steel packed with Spherisorb 10-μm ODS (Phase Separations, Clwyd, Great Britain) bonded reversed-phase material (5000 plates) and the mobile phase was[3] methanol–water (80:20) containing 13 g/l of 40% tetrabutyl ammonium hydroxide and 1.7% (v/v) orthophosphoric acid.

The flow-rate was set at 2 ml/min, and the internal standard used was 2,6-di-*tert.*-butylphenol. HPLC-grade solvent was obtained from Rathburn Chemicals (Walkerburn, Great Britain), tetrabutyl ammonium hydroxide and internal standard were supplied by Aldrich, Dorset, Great Britain.

RESULTS AND DISCUSSION

Fig. 1 shows the elution profile of a sample of α-, and β-acids, resulting from chromatography of a CO_2 extract taken up in methanol, with internal standard added. The standard eluted first, followed by the two α-acid peaks, the first consisting of co-

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humulone and the second of humulone and adhumulone. The β-acid peaks followed the same pattern, the first containing colupulone and the second lupulone and adlupulone.

Fig. 2 shows the result of injection of isomerised CO_2 extract, with the double peak of the iso-α-acid eluting prior to the internal standard. The first peak contained isocohumulone and the second isohumulone and isoadhumulone.

Chromatography of beer samples was carried out by adding internal standard at a lower concentration to beer and measuring at an expanded absorbance scale (Fig. 3).

Calibrations of iso-α-, α- and β-acid were carried out with purified samples in methanol, and for beer measurement the calibration was made in ethanol. Since the internal standard, made up in methanol, is not completely soluble in a dilute ethanol solution, this resulted in a slightly opaque solution, but injection of this solution had no effect on either the beer iso-α-acid or internal standard peak areas.

During the measurement of iso-α-acid in both extracts and beer, the appearance of a shoulder on the downslope of the second peak was always observed. This was taken to be due to the isoadhumulone present, and when the flow-rate was reduced to 1 ml/min the shoulder formed a separate plateau area from the larger peak, although it did not form a peak itself. This produced an error in the measurement of the iso-α-acid, since the integrator did not recognise the small isoadhumulone area as a peak and consequently did not measure it. Thus, retaining the flow-rate of 2 ml/min produced a faster chromatographic run and greater accuracy in the measurement of the iso-α-acids.

To enable measurement of iso-α-acid concentrations in a wide range of beers, it may be necessary to make a preliminary dilution of the sample to a standard ethanol concentration prior to addition of the internal standard and chromatography.

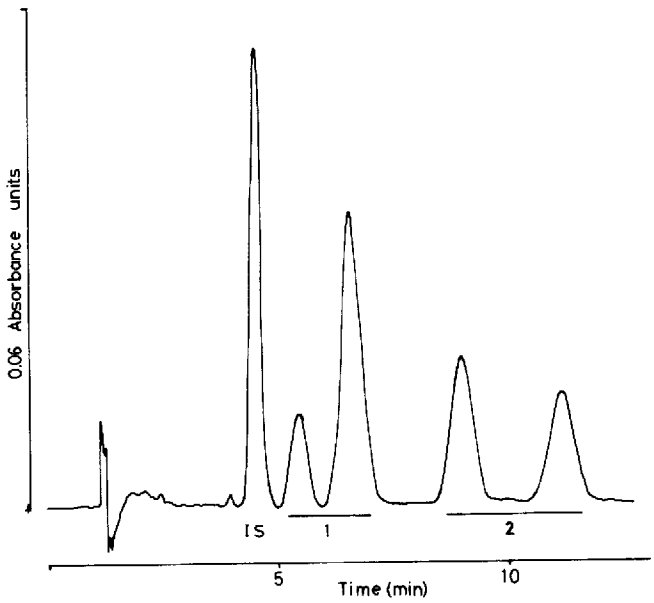
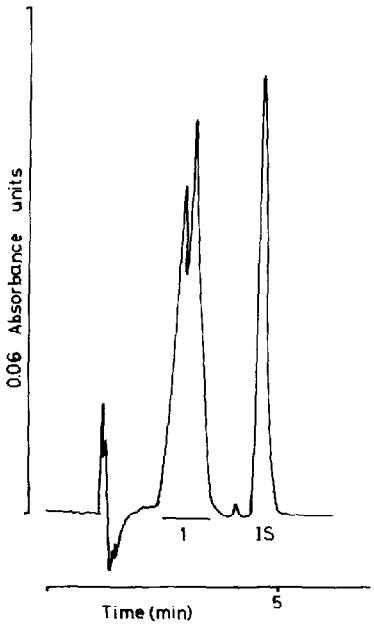


Fig. 1. Chromatogram obtained from injection of 20 μl of hop extract in methanol. For conditions see text Peaks : IS = internal standard ; 1 = α-acid ; 2 = β-acid.



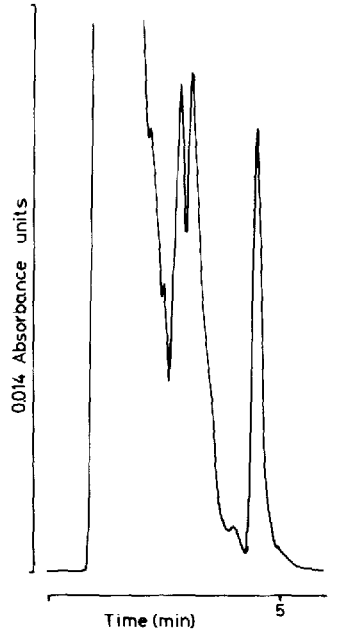


Fig. 2. Chromatogram of isomerised CO_2 extract under the same conditions as Fig. 1. Peaks : IS = internal standard ; 1 = iso-α-acid.

Fig. 3. Chromatogram of 20 μl beer under the same conditions as Fig. 1 except with an expanded UV absorbance range and addition of correspondingly less internal standard.

The method reported offers an attractive system of hop acid measurement in that all of the important acids in terms of bitterness can be measured on an isocratic system with a small injection-to-injection interval. Work is being carried out to compare Bitterness Unit values of bitterness with those of iso-α-acid concentrations.

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