International subcommittee for Isomerised Hop α-Acids Standards

Fourth Meeting of the International Subcommittee for Isomerised Hop α-Acids Standards

The Fourth Meeting of the International Subcommittee for Isomerised Hop α-Acids Standards was held in Keystone, Colorado, USA, on the 12th September 1999. Six full members (out of a possible twelve) were in attendance, plus one proxy member and two guests. Regrettably, on this occasion only three of the subcommittee's four parent organisations (ASBC, EBC, IOB and BCOJ) were represented, neither of the two committee members appointed by the Institute of Brewing having been able to make the trip.

Following acceptance of the Secretary's Report of the Third (Cannes) Meeting, the Chairman (Richard *Wilson*, S. S. Steiner, Inc.) promptly turned the meeting's attention to the continuing study of the purity and stability of small-scale preparations of the proposed new standards for the HPLC analysis of isomerised and reduced isomerised α-acids.

Elemental analysis

It will be recalled that, at the previous meeting, results were presented for the elemental analysis (C, H, O & N) of small scale preparations of DCHA-iso-α-acids, DCHA-rho-iso-α-acids and crystalline tetrahydroiso-α-acids. With one or two exceptions, the data had indicated close agreement with calculated values for the three compounds (and after taking account of the individual cohumulone ratios for each preparation). This meeting was now presented with the data for the fourth standard, DCHA-hexahydroiso-α-acids. Agreement was considered excellent for two of the four preparations, but for the other two it was noted that the oxygen value was not quite in line with expectations. In view of other data indicating a high purity for both these preparations, it was suggested that the incorrect oxygen values were due either to presence of a small amount of water and/or solvent residue, or else simply to error in the measurement. (In respect of the latter possibility, it was noted that the value obtained for oxygen content is less accurate than for carbon and hydrogen, since this value is calculated by difference, rather than being explicitly determined. Thus, any systematic errors in measuring C, H or N would have a disproportionately large effect on the derived oxygen figure). After concluding that, in all probability, the four small-scale preparations of DCHA-hexahydroiso-α-acids were each fundamentally of high purity, the meeting nevertheless agreed that it would be appropriate to check for absence of both water and solvent residues in future preparations of the proposed standards.

Storage stability

Attention was next given to the results of the previously organised trial to establish the stability in storage of a selection of the small-scale standards. Duplicate samples of these standards had been separately stored over a period of four months at freezer and ambient temperatures by each of the participating laboratories and then compared against each other by duplicate or triplicate HPLC analysis. Generally, the results were encouraging, although one or two laboratories appeared to have difficulties in obtaining consistent analyses. Of the four standards, DCHA-rho-iso-α-acids and DCHA-hexahydroiso-α-acids were considered to show no significant signs of differences that might imply instability. Tetrahydroiso-α-acid samples were reported as possibly unstable by one laboratory that compared its data with the results obtained four months earlier, but in view of a lack of chromatographic evidence of deterioration, it was thought most likely that the apparent loss of "tetra" was due to instrumental causes. Drawing conclusions regarding the stability of DCHA-iso-α-acids was considered to be hampered by a relative lack of data. Some evidence was seen for a slightly lower content of iso-α-acids in the ambient stored sample of one laboratory, but it was not sufficiently large to be considered of definite significance.

Overall, the results of stability testing were considered sufficiently encouraging to continue the trial. A second round of comparative analyses was to be conducted in December, 1999.

Preparation of the new standards

Agreement to proceed to the bulk preparation of the actual standards themselves was reported at the last meeting. Substantial progress towards achieving the target of producing all four standards was now reported by the three individual committee members who had graciously volunteered to undertake this onerous task.

DCHA-iso-α-acids

First, John Paul *Maye* (Haas Hop Products, Inc., ASBC) reported that he had prepared a batch of material in better yield than previously reported in his recently published paper (J. Am. Soc. Brew. Chem. 57, 2, 55 – 59, 1999) by switching to use of acetone as a solvent, but that this preparation had contained a small amount of contamination with three unidentified compounds that had shown up on HPLC analysis. Two of these compounds he suspected to be either cis-iso-α-acids or allo-iso-α-acids. His proposal to try to improve the purity of his preparation by re-crystallisation from ethyl acetate, which he knew would, at the very least, remove the third contaminant, was accepted by the meeting.

Crystalline tetrahydroiso-α-acids

Next, Gus *Gusinski* (Kalsec, Inc., ASBC) reported that he had made substantial progress with the preparation of the tetrahydroiso-α-acids standard and was confident that he had enough material to ensure production of the intended quantity of purified material. His problem had been to induce crystallisation of the

desired mixture of isomers and homologues. He now proposed to blend several batches of pure material having different compositions and then try to induce rapid crystallisation in the hope of producing a single, homogeneous material. This was agreed to be a better solution than to attempt to blend together the crystals of these batches. He would also attempt to check the homogeneity by selecting individual crystals for HPLC analysis by a method that separates the isomers and homologues.

DCHA-rho-iso-α-acids

Lastly, the Chairman presented the written report that he had received from Paul *Hughes* (Brewing Research International, IOB), which outlined the progress made regarding preparation of the DCHA-rho-iso-α-acids and DCHA-hexahydroiso-α-acids standards. This report indicated that two batches of the former standard had been prepared, but that in each case the quantity produced was insufficient to meet the previously targeted amount. In view of this shortfall, it was agreed that the Chairman should contact Dr. Hughes with a view to having these preparations combined and then recrystallised as a single batch.

DCHA-hexahydroiso-α-acids

Dr. Hughes' report also indicated that the DCHA-hexahydroiso-α-acids standard was prepared and was now ready for evaluation. It was noted, though, that elemental analysis showed a slightly low result for the oxygen content. Also, since he had not determined the cohumulone ratio of his preparation, it was not known whether the carbon and hydrogen values were correct, though they clearly fell within the absolute limits for DCHA-hexahydroiso-α-acids.

The meeting agreed that, once all four preparations were completed, it would be sensible for three or so samples of each to be distributed as appropriate to selected volunteers who had the ability to examine some or all of these preparations by HPLC. The objective would be to have each standard tested using two or more different methods so that the presence of any contaminant substances might be better established and isomer and homologue ratios established where not already known.

Dr. Richard J.H. Wilson, Chairman