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Dear Sir,

Hereby as promised the manuscript on our latest methods for hops and extract analysis. This text was sent to the J.I.B. editor for eventual publication.

Yours sincerely,

M. Verzele.

ON THE ANALYSIS OF HOPS BITTER ACIDS

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SUMMARY

A High Performance Liquid Chromatography (HPLC) method for automated routine analysis of the hops bitter acids is described. It allows the simultaneous determination of α -acids, β -acids, cohumulone and colupulone. This analysis based on two chromatographic runs takes less than 1/2 h. With this new method as reference, the defects of existing conductometric titration techniques were studied and a new improved conductometric method based on toluene-buffer extraction could be developed. The new methods are applicable to all forms of hop products containing α - and β -acids.

It is proved that α -acids analysis based on ether-acid aqueous methanol extraction gives too high results because of several basic defects pushing the α -acids % or lead conductance value upwards.

I. INTRODUCTION

Conductometry is the method mostly adopted for the analysis of α -acids. Analytica III (1) lists several conductometric procedures for hops and for hop extracts. None of these methods is completely satisfactory. Their main drawback is lack of selectivity, insufficient accuracy and the fact that there is no method equally applicable to all forms of hops and hops derived products. Still, these methods are used as the main

quality control factor for α -acids containing products. Recently, the method developed by us before (2) has been criticised (3) and its replacement by another conductometric procedure has been suggested (4). This prompted us to reexamine α -acids analysis once again.

It is evident since a long time that what is needed for α -acids analysis is a reliable reference method. At one time Counter Current Distribution (CCD) was considered to be acceptable as reference (5) but in practice this turned out not to be the case. The more recent ion exchange chromatographic method of Otter, Silvester and Taylor (6) showed promise, but ring analyses, organised both by EBC and ASBC were not entirely satisfactory. Slightly modified versions of this method seem to be better and are under investigation. A definite drawback of this soft gel ion exchange chromatographic α -acids analysis is the long analysis time involved and resolution could be bet

It is obvious to date, to turn to HPLC for the reference problem and several authors have already tried to do so (7,8, 9,10). It cannot be stated however that any of these published procedures is up to the standards of modern HPLC on the points of accuracy, resolution, speed and accessibility of column material. In a recent paper (11) we described superior systems for the HPLC of hop bitter acids. One of these resolves cohumulone, adhumulone-humulone, colupulone and lupulone-adlupulone from other compounds eventually present. The internal standard was β -phenylchalkone. We have now slightly changed the eluting solvent so that the more readily available chalkone can be used as internal standard. A typical chromatogram is

shown in fig.1. The chromatographic run takes only 10 minutes and the sample preparation is extremely simple and foolproof. This method will now be discussed in detail.

II. HPLC ANALYSIS OF α -ACIDS

1. Instrumentation and chemicals. Needed :

- a HPLC instrument capable of working at least at 250 kg/cm² and delivering a constant solvent flow (we worked with Varian 8520 and Varian 5020 LC instruments)
- a sample loop injector delivering 10 μ l of the sample solution (we worked with a Valco 7000 psi injector. Syringe stopped flow injection is also possible but this will shorten column life and needs experience)
- a variable wavelength(UV) detector capable of measuring at 344 nm (we used a Varichrom detector - precise calibration of the detector is very important)
- a 25 x 0.46 cm octadecyl bonded 10 μ silicagel column. This reversed phase should contain 15 to 20 % organic material; it must be acid treated to remove iron. We used RSil 10 μ SiO₂-C₁₈ (17 %) (12)
- solvents like toluene, methanol and water. They have not to be of special grade quality
- 2,6-di-t.butyl-4-methylphenol or so called butylated hydroxy toluene (BHT), a well known anti oxidant
- phosphoric acid 85 %
- chalkone purified by crystallisation in iso-octane till the melting point is 57-58°

- 0.1 M primary-secondary phosphate buffer pH 6.0

2. Sample preparation

- for hops, hop powder, hop pellets : 10 g is shaken for 1/2 h with five 1 inch glass marbles in 100 ml toluene containing 1 % of BHT. A portion of the mixture is centrifuged in a stoppered tube for a short time (1-2 min). 2 ml of the clear solution is evaporated on a rotavapor and with a pipet 50 ml (or 100 ml if α -acids is presumably above 7-8 %) methanol is added, containing 0.25 % H_3PO_4 à 85 % and 1 % BHT and an exactly known amount of about 15 mg/l chalkone as internal standard
- for hop extracts 50 to 100 mg of the extract is weighed on a polythene foil and placed in 100 ml methanol containing 0.25 % H_3PO_4 à 85 % 1 % BHT and again 15 mg chalkone per litre as internal standard.

If possible the amount of extract sample is adapted to the presumed α -acids content : ~ 50 mg for 40 % α -acids, ~ 100 mg for 15 % α -acids.

The solvent volumes chosen in the above procedures are rather large (100 ml toluene and 50 ml methanol for hops and 100 ml methanol for extracts). The reason for this is to minimise the "dilution factor". Toluene will extract about 25 to 30 % of the sample of hops, hop powder or pellets. Dissolved in 100 ml of toluene this extract of a 10 g sample will increase the solution volume by 2-3 % thereby decreasing the end α -acids result by the same amount. In the end calculation of the analytical result this error could be taken into account by mul-

tipling with the factor 1.02. This "dilution factor" error is small against the large errors inherent to α -acids analysis in hops, powder and pellets. By first dissolving extract in 10 ml methanol containing standard and then diluting 1 ml to 10 ml, the analysis could e.g. be run with only 20 ml of methanol. In this case however there is a "dilution factor" error of -0.5 to -1 %. It is better to avoid this as the accuracy of the α -acids analyses in hop extract is high enough; therefore the large solvent volume. This "dilution factor" error is more fully discussed further in this paper. The measuring wave length of 314 nm is high enough so that the methanol and toluene have not to be "spectroscopically" pure solvents. A once distilled industrial quality will be sufficient. The cost of these, even in the larger amounts is negligible.

3. Chromatography

The loop injector is loaded with 10 μ l of the sample solution, using a freshly cleaned syringe. Beware of metal when working with α -acids ! Stainless steel is more or less acceptable, but iron or steel is deadly - the plunger of most small sample syringes is in steel ! Use a glass plunger syringe or one with a teflon tipped plunger - the needle must be in stainless steel or even platinum. The analysis is run isocratically with a solvent mixture of methanol-water-phosphoric acid 85 ml / 17 ml / 0.25 ml containing 1 g of BHT. The solvent rate is set at 120 ml/h and the detector at 314 nm. The sample is injected and the chromatogram will be ready in about 10 minutes.

Pressure during the chromatographic run was typically around 200 kg/cm². With such fast chromatography it is convenient to run the same analysis 2 times. This will of course increase reliability of the mean result. Standard analytical practice requests indeed two concording results.

Full instrumental details in our case were as follows :

Recorder : Varian A25, chart speed 1 cm/min, full scale span 1 mV, integrator : Varian CDS-111, attenuation 8, the programme was :

	Section 1
1 ID=	0=
2 S/N	4N
3 IPW	12S
4 TAN %	3.20 %
5 AREJ	5ØA
6 STOP	ØØM

Detector : Varian Vari-Chrom, absorbance range : 1, band width : 8 nm, time constant: normal, sample cell position : front.

4. Computation of percentages

When using an integrating computer the area percentages of all peaks will turn up automatically at the end of each chromatogram. Calculation of the percentages of α -acids, cohumulone, adhumulone-humulone, β -acids, colupulone and lupulone-adlupulone is based on these chromatographic area percentages, the extinction coefficients of the compounds and on the calibration versus the internal standard chalkone.

Against this internal standard a series of increasing weight samples of pure humulone gave a straight calibration line

over a concentration range of 3 to 20 mg humulone per 100 ml HPLC solvent (13). The equation for this line is

$$y = 0.1753 \cdot x + 0.0076$$

with a correlation coefficient of 0.99984

y is the ratio of surface areas of humulone over that of chalkone and x is the ratio of weights. The values of the surface areas are printed by the integrator. To obtain the value for cohumulone, the correction factor is the ratio of extinction values of humulone over cohumulone or $208/198 = 1.050$. Adhumulone and humulone are not separated in this HPLC system; at 314 nm they have the same extinction coefficient. α -acids is then the sum of the peaks for cohumulone and for humulone-adhumulone. An example of the calculations for hop extracts is shown in table 1 :

Table 1

I Sample	II Surface chalkone	III Surface cohul.	IV Surface humul. + adhum.	V IV/II	VI III x 1.050
64,18 mg in 100 ml solvent. Chalkone 0,014043 mg/ml	1707	1760	4009	2,3485	1848
=====					
VII VI/II	VIII VII + V is y	IX x as calc.	X 0,014043.x or weight α -acids	XI dilution factor 100	XII α -acids % calc.
1,0826	3,4311	19,5228	0,2741	27,41	42,71

In other words : x is calculated with the regression equation

$$\text{and then } \% \alpha = \frac{x \cdot \text{mg standard} \cdot 100}{\text{mg hop extract}}$$

For hops it is as if 400 mg were dissolved in 100 ml methanol and the calculation is then, taken the dilution factor into account : $\% \alpha = \left(\frac{x \cdot \text{mg standard} \cdot 100}{400 \text{ mg hop}} \right) 1.02$.

Integration based on peak heights and retention time, or on peak width at half peak heights are not applicable, as the columns gradually deteriorate and produce changing chromatograms. To calculate separately cohumulone or colupulone content is straightforward; instead of adding the surface areas, each is calculated in % individually.

5. Extinction coefficients of the bitter acids

In a recent paper (14) the extinction coefficients of many of the bitter acids were published and discussed. The HPLC solvent system used in the present paper is however again different and the extinction coefficients have to be determined once more. In the methanol-water phosphoric acid-BHT system the three major α -acids have as extinction coefficient at 314 nm : humulone-adhumulone 208 and cohumulone 198.

Of the β -acids only colupulone could be obtained pure and the extinction coefficient was measured directly. Chromatography of a weighed amount of colupulone against chalkone and also of total β -acids against chalkone allows the calculation of the % colupulone in β -acids. Extinction measurements of the β -acids mixture with known composition leads to the specific extinction of the lupulone-adlupulone mixture.

The values are : colupulone 163; lupulone-adlupulone : 174.

β -acids percentages can be calculated in the way as above for α -acids using the same chromatogram. The "corrections" or ratio of E values is now 1.28 for colupulone and 1.20 for lupulone-adlupulone.

6. Wave length and wave length calibration

At 314 nm the photometric absorbance is at or near a maximum for both α -acids and β -acids. A deviation of only 1 or 2 nm on the wave length selector of the HPLC-UV detector will still cause serious errors. Wave length calibration is therefore absolutely essential. Monitoring the detector (with a 2 nm slit) against the very sharp absorption maximum at 311 nm of naphthalene in isooctane will show if resetting is needed. To do this a solution of naphthalene in isooctane having an extinction of 0.5 to 0.8 is placed in the detector cell. The detector wave length selector is set at 305 nm and the recorder pen at about 3/4 deflection. The recorder is allowed to run for about 1-2 cm, stopped, the wave length selector set at 306 nm and the operation repeated till about 317 nm. The step heights against wave length on a graph will produce an inverted peak which should be at its lowest at 311 nm. If this is not so the detector needs resetting. This was the case with our instruments. This wave length check should be repeated occasionally. An important remark is that the analyses can be and were therefore run with the visible lamp light source of the detector. UV lamps need relatively frequent replacement and are rather expensive while the visible lamps are cheap. This is therefore an interesting economic point.

7. The HPLC column

α -acids are very sensitive to metal traces as has already been emphasised. We have studied metal impurities in several commercial silicagels; none is completely metal or iron free. There are however large differences. In fact α -acids chromatography without efficient complexer is a very good test for this aspect of octadecyl silicagel quality. With only traces of accessible iron in the material the α -acids percentage will still be too low although the chromatograms look practically normal. The effect of silicagel bonded iron can be blocked by saturating the column with α -acids, with phosphoric acid or with EDTA. Rinsing with eluting solvent without phosphoric acid reestablishes the base line and the column can then be used for a few analyses without trouble. The column activity comes back soon however and we obtain the original situation after some time. The addition of iron salts to the eluting solvent results in low α -acids percentage. This iron in solution can also again be rinsed from the column with solvent.

To improve octadecyl-bonded silicagel for α -acids analysis, the interfering metal ions have to be removed from the starting silicagel material by very careful and repeated acid treatment followed by extensive washing. Curiously enough and in contradiction with a general belief, octadecyl silicagel itself can also be boiled with 1 N HCl without destroying the phase. Such treatment improves α -acids chromatography but not to the required level. Details of our studies on this particular

problem will be published elsewhere (15). One way around the problem would be to chromatograph in the presence of EDTA. Solutions of 0.5 to even 1 % of sodium or potassium salt of EDTA can be made in the eluting solvent without apparent trouble. With time, EDTA itself starts to precipitate as very fine crystalline material and this blocks filters and the HPLC column. To avoid this trouble the EDTA concentration can be only 0.01 % and then this is not longer completely effective. Glycine was also tried as iron complexer. This has better solubility than EDTA but is only partly effective. Phosphate is also a well known complexer for iron but addition of some potassium biphosphate changes the chromatographic pattern drastically. Finally then, phosphoric acid turned out to be a satisfactory complexer while still producing good chromatograms. It is possible to demineralise reversed phase silica-gel in such a way as to obtain correct results without adding complexers. This is extremely difficult however. Therefore whatever the origin of the phase it should be boiled three times for 2 h in methanol/2 N HCl - 60/40 and the α -acids chromatography should be run with about 0.25 % phosphoric acid in the eluting solvent. The above remarks on the optimization of α -acids HPLC are condensed in table 2.

Table 2

10 μ SiO ₂ -C ₁₈	Treatment	% α -acids
1. Self made insufficient deironing	none	~10-15
2. Idem as 1	0.01 % EDTA in solvent	28.0
3. Idem as 1	boiled 3 times with 1 N HCl	39.1
4. Idem as 1	with 0.25 % H ₃ PO ₄ as complexer	39.1
5. RSil (RSL)	none	37.6
6. Nucleosil (Macherey Nagel)	none	38.2
7. Lichrosorb RP 18 (Merck)	none	31.5

Table 2 : α -acids % of hop extract as found by HPLC. With 0.25 % H₃PO₄ as complexer in the eluting solvent, all the commercial octadecyl silicagels of table 2 give correct results.

8. General discussion and results by HPLC method

In our previous publication on the HPLC of hop bitter acids (11) we advocated the use of β -phenylchalkone as internal standard and the solvent system was methanol-water-HAC in the ratio 85-15-1 volumes. Addition of BHT as now introduced changes resolution somewhat. Furthermore we changed acetic acid to phosphoric acid and also slightly changed eluting solvent composition. In doing so it became apparent that chalkone is then also sufficiently separated to be possible as internal standard.

Chalkone has the advantage that it is easily obtainable. It can be purified by recrystallization from isooctane. It should have a m.p. of 57-58°. Chalkone has the disadvantage that it is photo isomerized by day light into a mixture of trans and cis forms. This drawback can be avoided by simply keeping the chalkone solutions in the dark (aluminium foil). Trans chalkone in these conditions is stable over a very long period. The influence of BHT addition was studied extensively. Oxidation of α -acids seems not to occur every time a sample is processed, even with hop samples which are more readily oxidised than hop extracts. If there is no oxidation, BHT is not necessary. It does not interfere in the chromatography or conductometry of α -acids and will block eventual oxidation of α -acids. Therefore it is added to all solvents used in the method.

One question that should be raised is : what about other, minor α -acids which could be separated by HPLC and thus escape analysis while being titrated with lead ? Post- and prehumulone can probably be separated from the major α -acids by HPLC. Research on this may need several years of application of the HPLC method to ascertain the importance of this factor. Only experience will tell the frequency (if ever) of hops or extracts turning up with high post- or prehumulone content. As a start we analysed the lead precipitate of various extracts used in this study. These lead salts were purified by washing and reprecipitation as carefully as possible. A trace of such an analysis is shown in fig. 2A. There is practically nothing else to see but the peaks for cohumulone and combined humulone-adhumulone. Better or a different resolution of α -acids can

be obtained with HPLC using a buffer of pH 7.0 as eluting solvent. A trace in these conditions is shown in fig. 2B. Adhumulone is now separated and 3 minor peaks can be seen. By increasing recording intensity the trace of fig. 2C is obtained. Next to the 3 major α -acids at least 8 peaks can be seen. It is doubtful that these are all α -acids. Their surface area amounts to 1-2 % of the major α -acids peak area. In the acidic conditions of the proposed analytical method these extra peaks are not revealed.

With hop extracts, the sample preparation is no problem. The amount to weigh being small, good mixing of the extract must be emphasised.

For hops there is the point which solvent to prefer. With the HPLC method it is possible now to study this with confidence and to analyse precisely what time is needed for complete extraction. To do this, a sample of ground hops was shaken with solvent and five 1 inch glass marbles and the solution was analysed after 10, 20, 30, 60 and 120 minutes. We used methanol (polar) toluene (apolar) and ether (in between polarity) and it turned out that extraction is equally rapid with the three solvents. We did these experiments with and without BHT addition. To our surprise oxidation of α -acids can occur more easily in methanol than in toluene. For β -acids the reverse is certainly true.

One point about hop analysis which was now made very clear, is that the largest cause of error is sample inhomogeneity. With some hops this factor is only of minor trouble but with

others or even with most samples it causes very large errors. One way around this problem could be to use very large samples. Series analyses with 30 g hop samples showed however only slight improvement. Some results are shown in table 3.

Table 3

Sample weight	Conductometric	HPLC
5 g	-	8.4
5 g	8.3	8.4
5 g	7.5	7.9
10 g	7.6	8.0
10 g	7.8	8.1
30 g	8.1	8.6
30 g	-	8.5
30 g	7.9	8.2
30 g	8.1	8.6
30 g	8.5	8.8
30 g	8.2	8.4

$\Delta C-HPLC$
 -0.5
 -0.3
 -0.5
 -0.3
 -0.2
 -0.36 \pm 0.13
 -0.90 \pm 0.13
 8.16 \pm 0.22
 2.7% CV
 8.52 \pm 0.20
 8.33% CV

Table 3 : α -acids determination on 1978 hop sample circulated by EBC for comparative analysis. Note that for this fresh hops the HPLC results are generally higher than the conductometric results.

We believe now that precise α -acids analysis in hops is impossible. This assertion is not only based on the present study, but comes also from long experience with many different methods, from many different ring analyses and interla-

boratory exercises, all showing that a realistic relative variance of 5 to 10 % has to be accepted for hops analysis. It is most imperative that all parties involved in hop transactions become aware of this fact.

With hop extracts the situation is much better as extraction is of course a strong homogenizing factor. The same situation prevails with other, e.g. conductometric analysis methods. For the HPLC method at hand the relative standard variation based on 10 analyses was about 1.5 % on the Varian 8520 instrument and on the Varian LC 5020 instrument. An example of how results look like in comparison with the improved conductometric procedures as detailed further is shown in table 4.

Table 4

Sample	Conductometric α-acids	cohumulone	humulone adhumulone	α-acids	colupulone	lupulone	β-acids
Standardised (EBC)	21.5	6.6 (31%)	14.7	21.3	5.4 (53%)	4.8	10.2
Pure resin (EBC)	43.4	13.4 (31%)	30.5	43.8	10.7 (54%)	9.1	19.7
Pure resin (PRB)	43.5	13.6 (31%)	29.7	43.4	11.4 (54%)	9.8	21.2
Pure resin (PRB)	39.4	12.1 (31%)	27.0	39.1	9.3 (53%)	8.1	17.4
Standardised (PRB)	20.1	8.0 (40%)	12.3	20.3	4.8 (66%)	2.3	7.2

Table 4 : α-acids % determination on hop extracts. EBC samples circulated in 1978 for comparative analysis. PRB commercial samples. Optimised conductometric and HPLC methods as described in the paper.

This table shows also the detailed information which is obtained by the HPLC method. The first sample is a 50/50 dilution of the second sample. Samples 1 to 4 are probably all obtained from the same hop variety (Northern Brewer ?) while sample 5 is obtained from a different sort of hops as evidenced by the different α - and β -acids composition.

III. CONDUCTOMETRIC α -ACIDS ANALYSIS

What is wrong with the existing conductometric methods ? A number of points can be mentioned, more or less important according to the particular procedure. The most pertinent are :

1. The conductometric titration

There is something wrong with the conductometric titration of α -acids. The titration of pure humulone in methanol gives variable and generally low results. For a long time we have attributed this to impurities in the humulone that could not be avoided. This can however not longer be accepted now that HPLC proves the purity of the humulone used. Indeed the titration result goes up and becomes more reproducible by addition of DMSO or of pyridin. This particular point was already mentioned extensively in our paper of 1971 (2) but somehow it has not received sufficient attention. A clear indication about the difficulties of the conductometric titration is the fact that back titration gives a higher result than the direct titration. By adding excess lead acetate and on back titration with sulphuric acid the resulting α -acids or humulone % figures are indeed higher by about 0.5 to 2 % in our laboratory.

In another laboratory these figures were even about 4 % higher. Back titration with oxalic acid leads to the same constatation. This discrepancy between direct and back titration results for lead acetate is not reserved to α -acids; with sulphuric and oxalic acids the same observations can be made. How to be sure in such conditions about the normality of the lead acetate solution ? We tried to purify lead acetate by repeated recrystallization but without change in the results. We also tried to replace lead by silver and magnesium; equally without result. We finally decided to use the titer found with lead acetate in the burette and sulphuric acid below because this is the way α -acids are titrated and because these results agree best with the HPLC figures. We have no explanation for the basic anomaly.

2. The dilution factor

When an organic chemical mixture like hop extract is dissolved, the volume of the solution increases. This we checked with 20 ml toluene, ether or methanol in a burette by adding a known amount of extract : the volume increase is equal to the volume of the extract dissolved. In our toluene/buffer method (2), the sample for a rich hop extract is about 250 mg to 500 mg. The corresponding volume increase of the 20 ml toluene layer is thus about 1 to 2 %. This depresses the end α -acids percentage result by an equivalent percentage. For a 10 g hop sample extracted with 100 ml of toluene and containing 20-30 % extractable organic material, the error caused by this factor is of the order of -2 -3 %.

Analytical figures proving this particular point for hop extracts are collected in table 5.

Table 5

Increasing amount of hop extract	1	2
Sample A		
± 500 mg	31.5	35.8
± 900 mg	31.2	<u>36.1</u>
±1200 mg	30.8	35.5
±1500 mg	30.7	34.8
Sample B		
± 600 mg	29.6	34.0
±1300 mg	28.0	33.4
±1600 mg	<u>28.2</u>	33.1
	<hr/>	<hr/>
	Σ210.0	Σ242.7

Table 5 : Conductometric α -acids analysis in function of sample size. Column 1 : "Verzele" method as described in reference 2 and criticised in reference 3. Column 2 : "Ganzlin" method, see reference 4.

The gradual decrease of α -acids content with increasing sample size has been pointed out as a major defect of our toluene/buffer method (2,3) but it also occurs with the method advocated to replace it (4) ! Note the difference in result between the toluene/buffer results of row 1 and the results with ether/acid aqueous methanol method of row 2.

That there are some figures (underlined) not conforming to the general pattern is only natural considering the precision of the analysis. Nevertheless the general tendency, proved by many series of similar experiments, is clear.

The difference between the two series of results is of the order of 15 %. This is very high but it has been confirmed by other laboratories that the difference between the two methods is indeed between 6 to 15 %. The reasons for this are discussed further. Because of the dilution factor, analytical methods using a larger solvent volume for the α -acids analyses are therefore to be preferred.

3. The two phase distortion factor

Easy dissolving of all types of hop extracts needs a two phase solvent system in which one is an aqueous phase. One criterium of the solvent system chosen must be that the volumes of the phases are not changed on mixing. This is the case for the solvent system toluene/water (buffer) but it is not so for the system ether/acid methanol-water of the Ganzlin method (4). It is well known that ether dissolves in water and even better in methanol/water. Water dissolves in ether too and whether the all over effect at room t° will be a volume increase or decrease of the ether phase has to be checked experimentally. This is most easily done as follows : in a 50 ml burette 15 ml of a mixture methanol (5 ml) water 0.1 N in HCl (10 ml) is introduced and the burette reading is noted. 25 ml of dry ether is now pipetted in the burette which is

closed on top with an efficient rubber or cork stopper. After sufficient shaking it can be noted by burette reading that the volume of the aqueous layer has increased and that the volume of the ether layer is not longer 25 ml but is reduced by about 2-3 %. With undried ether this figure is even higher (3 to 4 %).

The bitter acids, being exclusively in the ether phase as ascertained by HPLC, are therefore concentrated because of this factor for about 2-4 %.

For this reason ether must be avoided as solvent in a two phase hop extract analysis.

4. Solvent evaporation

Evaporation of the organic solvent during the analytical procedure will increase the concentration of bitter acids and the result will be higher than it should be. With the necessary precautions, evaporation of toluene or methanol e.g. is negligible. Not so with ether. Taking all precautions as recommended in the ether/methanol-acid water method (4) and although room temperature was only 20° it can be easily shown experimentally that ether evaporation causes an increase in the end result.

To measure this we used a naphthalene solution in ether showing an extinction value at 276 nm of 0.650. After using this ether in a dummy run of the conductometric α -acids analysis (without adding the aqueous phase !) the extinction value had increased to 0.656. This increase, due to evaporation is therefore of the order of 1 %. Being less careful and in hot

weather this figure can easily be much larger.

The combined effect of III.3 and III.4 is shown in the following experiments. With a rather pure extract (~ 40 % α -acids) it was possible to carry out the conductometric titration directly without doing the two phase partitioning and the rest of the complete analytical cycle. The results of the comparison are found in table 6.

Table 6

1 Extract amount in mg	2 Directly	3 Complete procedure
~ 500	42.3	44.0
~ 800	42.3	43.6
~ 1200	41.8	43.1
~ 1500	40.9	42.9
	<hr/> 167.3	<hr/> 173.6

Table 6 : Column 1 gives the approximate amount of extract used in the determination. Column 2 are the results for the direct titration after dissolution in ether. Column 3 are the results for the complete Ganzlin procedure.

The figures in column 3 are ~ 4 % higher than in column 2. This difference is due to points III.3 and III.4.

Table 6 illustrates also point III.2 which is equally valid for both conductometric procedures under discussion : the value decreases with increasing sample size.

5. Hydrochloric acid in the ether phase

The lone oxygen electron pairs in ether form oxonium salts with acids and hydrochloric acid is therefore soluble in ether. The precautions taken in the recommended procedure to eliminate this hydrochloric acid are based on evaporation. This is a critical step and the slightest inattention can be the cause of trouble. Even so, with all precautions carefully respected, there are traces of hydrochloric acid in the end titration mixture. This can be shown by adding alkali at the appropriate step, mineralization and classic chloride detection with silver nitrate. The amounts of chloride are very small if all steps are carried out very carefully; the danger is however obvious. We therefore believe that hydrochloric acid should be avoided in the conductometric analysis of hop extracts. That sulphuric acid can cause trouble is known and it has been suggested to eliminate this by a solid sodium sulphate column filtration.

6. Emulsions

One of the drawbacks of our earlier conductometric method (2) turned up to be the formation of very strong emulsions with some extract samples. Although this occurred only rarely it was annoying. There are several ways around this particular problem. Instead of a buffer, an acid aqueous phase could be used for the dissolution of the extract sample. The buffer cleaning step could come afterwards. This approach does indeed work very well. It is however still easier to increase the volume of toluene relative to the buffer volume and even the

most difficult samples behave then properly. This larger toluene volume is also beneficial with respect to point III.2.

7. Buffer extraction of organic phase - Removal of α -acids

In our earlier toluene-buffer method, pH 7 was chosen, because experimentally we could not show the presence of α -acids in the material extracted with the buffer up to this pH. This was back in 1970 before HPLC. Now it can easily be shown that the pH 7 buffer extracts α -acids from the organic phase. Lowering the pH does help, but even at pH 5.5 the aqueous phase still contains traces of α -acids. The amount of α -acids extracted in the buffer also depends on the sample size of hop extract and on the volume ratio of toluene/buffer. Some HPLC data showing this are collected in table 7. This analysis of α -acids which are really dissolved in the buffer is rather difficult. There is some toluene phase dispersed in the buffer phase and repeated centrifugation and removal of the toluene traces with a mini pipette is necessary. Characteristic in this investigation is that the composition of the buffer dissolved α -acids has shifted strongly in favour of cohumulone. With toluene/buffer 50/20 the amount of α -acids extracted in the buffer is decidedly less than in the 20/20 procedure. How effective removal of oxidation products is has still to be studied.

Table 7

Sample weight	Buffer pH	Toluene Buffer 20 ml/20 ml	Toluene Buffer 50 ml/20 ml
x	7	0.32	0.10
3x	7	0.26	-
2x	6.5	0.18	-
2x	6.0	0.09	0.02
2x	5.5	0.02	-

Table 7 : α -acids in % on extract weight for a \pm 40 % extract as found in the buffer phase of the toluene buffer technique for conductometric analysis.

8. Buffer extraction of organic phase - Removal of oxidation products

Some oxidation products of α - and β -acids consume lead in the conductometric analysis. This was shown in our earlier publication and the point is still valid. Whether we should try to remove part or all of the oxidation products and aim for a true α -acids determination or whether we should do a lead conductance determination and call the result as such, is a delicate and debatable question. It is still not known whether the contribution of oxidation products of α -acids to beer bitterness is a positive or negative factor. In some cases for well handled hops or hop extracts this oxidation material titratable by lead may be small, but in other cases quite large amounts can be present. In our earlier paper (2) for a series of extracts, the difference with and without

buffer extraction was 7 %. We believe that more detailed and specific analysis is desirable and will help to solve some of the problems mentioned in this paper. Nevertheless, in order to avoid α -acids removal by the buffer we now believe a buffer with slightly lower pH should be chosen and pH 6.0 seems a good compromise.

9. New procedure for conductometric α -acids analysis

a) For hop extracts

The hop extract is mixed thoroughly before analysis and 250-400 mg for a presumably rich extract or 500-900 mg for a presumably poor extract are weighed on a polyethylene foil. This is placed in a 80 to 100 ml centrifugation tube together with 50 ml toluene containing 1 % BHT and shaken for a few minutes. 20 ml buffer pH : 6.0 is added and the well stoppered tube is shaken in a mechanical shaker for 10 minutes. The tube is centrifuged and 20 ml are pipetted in a beaker containing DMSO (10 ml) and methanol (10 ml). The conductometric titration and the graphic determination of the titration end point are carried out as in all previous techniques. Calculation of the α -acids % is then

$$\% \alpha = \frac{\text{ml PbAc}_2 \cdot 50 \cdot \% \text{ titer PbAc}_2 \cdot 357.8}{\text{g weight extract} \cdot 20 \cdot 379.3}$$

This new method avoids emulsion trouble, does only remove traces of α -acids by buffer extraction, minimises dilution factor errors and still maintains the advantages of speed and simplicity characteristic of our earlier procedure. The results are decidedly higher with the new than with the former method.

In general the figures compare very well with the HPLC results. The conductometric lead value is then practically equal to α -acids percentage. We believe this is a desirable situation. The above conclusions are illustrated in table 4.

b) For hops

10 g of hops, hop pellets, milled hops, hop powder etc., are placed in a plastic container of 250 ml together with five 1 inch glass marbles and 100 ml toluene containing 1 % BHT. The container is shaken rather vigorously for 30 minutes. Sufficient liquid is poured in a closed centrifugation tube and centrifuged for a short time. 25 ml of the clear toluene solution are pipetted in a 80-100 ml centrifuge tube together with 10 ml pH 6.0 buffer. The shaking period with the buffer can be reduced to a few minutes. 10 ml toluene layer are pipetted in a beaker containing DMSO (10 ml), methanol (10 ml) and toluene (10 ml). From this point on the analysis proceeds as for extracts.

Some results for older hops with this method are given in table 8.

As stated before, it is unrealistic to hope for good precision in hop analysis. Nevertheless, it seems that older hops give a higher conductometric than HPLC result (see table 8). This of course can be attributed to oxidation products. Why fresh hops give a higher HPLC result (see table 3) is however not so clear.

Table 8

Sample	Conductometric	HPLC
Australian 1976	3.5	3.4
unknown	2.7	2.3
unknown	3.3	2.9
Saaz 1972	1.3	1.0
Very old	0 ?	0.1

Table 8 : α -acids determination on old hops. Conductometric
toluene 50 ml/buffer pH 7.0, 20 ml HPLC normal. Even
with buffer pH : 7.0 the conductometric results are
much higher than the HPLC results. For very old hops the ti-
tration curve is unusable.

IV. SOME COMPARATIVE RESULTS

To conclude the paper some results for hop extracts, com-
paring the old and new conductometric procedure with the HPLC
method are shown in table 9.

We feel that the correlation between the two new methods is
good. Some results for fresh hops are shown in table 10.
Again, the difference in results is much larger and HPLC
tends to give higher figures.

Table 9

Sample	cond. pH = 7 20 tot/20 buf	cond. pH = 6 50 tot/20 buf.	HPLC -α	cohum.	colup.	β total
PRB 52730 Northern Brewer - 12/4/78	38.4 %	38.6 %	39.1 %	12.1 % 30.9 %	9.3 % 53.5 %	17.4 %
EBC hopextract (standardised 50/50 resins/tannin extract 3/10/78	20.4 %	21.5 %	21.3 %	6.6 % 30.9 %	5.4 % 53.2 %	10.2 %
EBC hopextract (pure resin) 3/10/78	42.1 %	43.4 %	43.8 %	13.4 % 30.5 %	10.7 % 54.2 %	19.7 %
PRB extract 93NB 10/11/78	41.7 %	43.5 %	43.4 %	13.6 % 31.3 %	11.4 % 47.2 %	21.2 %
PRB extract 2804 10/11/78	18.8 %	20.1 %	20.3 %	8.0 % 39.6 %	4.8 % 67.3 %	7.2 %
Hopulex - 1968	22.9 %	22.9 %	24.0 %	9.9 % 41.1 %	11.8 % 61.7 %	19.2 %
	Σ 184.3	190.0	191.9			

Table 9 : Analytical results : comparing conductometric with 20 ml toluene-20 ml buffer pH 7.0, conductometric with 50 ml toluene-20 ml buffer pH 6.0 and HPLC as described in the paper.

Table 10

Sample		Conducto- metric	HPLC			
			α -	% cohum.	β -	% colup.
Northern Brewer	1978	9.8	9.5	29.7	3.8	51.3
Brewers Gold	1978	7.0	7.3	47.1	3.8	69.5
Hallertau	1978	4.9	5.5	26.0	4.7	41.4
Saaz	1978	4.9	5.3	27.2	4.5	44.2
Star	1978	7.2	8.1	30.0	2.3	52.1

CONCLUSION

The work described in this paper has taken us nearly two years of continuous effort. We estimate to have run more than 1000 HPLC analyses on hops or extracts. The HPLC method is not only a reliable reference method but it is also a fast routine method. A HPLC instrument of another company than the one used in this paper, or even two similar instruments, may require a significantly different calibration equation. Each instrumental set up has therefore to be calibrated with either pure humulone or with an extract of known composition (13). Establishing a calibration curve as described in the paper takes about half a day time. Equipment cost is another drawback, but this is the way things are changing.

A similar challenge as the α -acids analysis is the iso- α -acids analysis in extracts, worts and beers. We are working on this.

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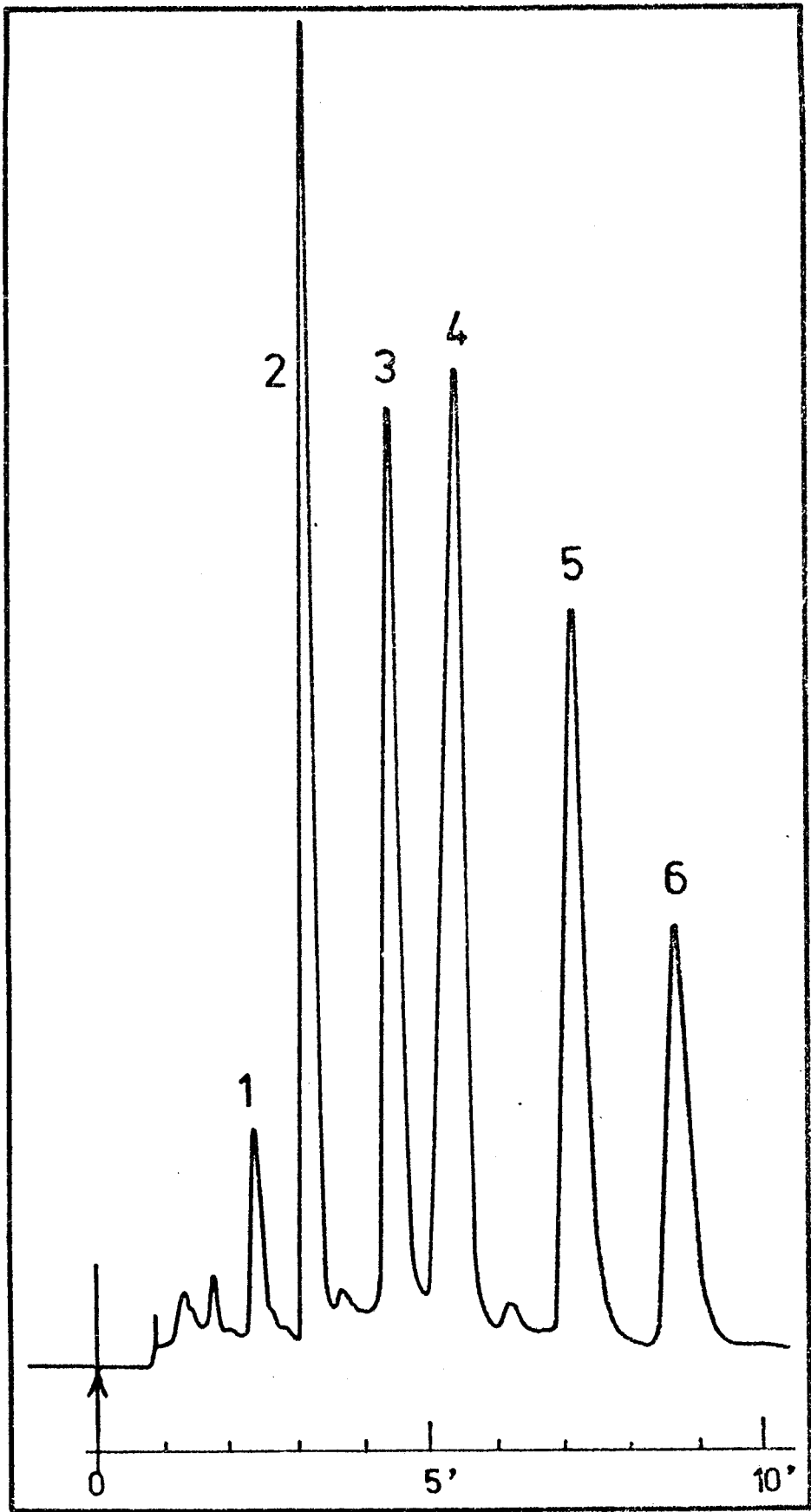
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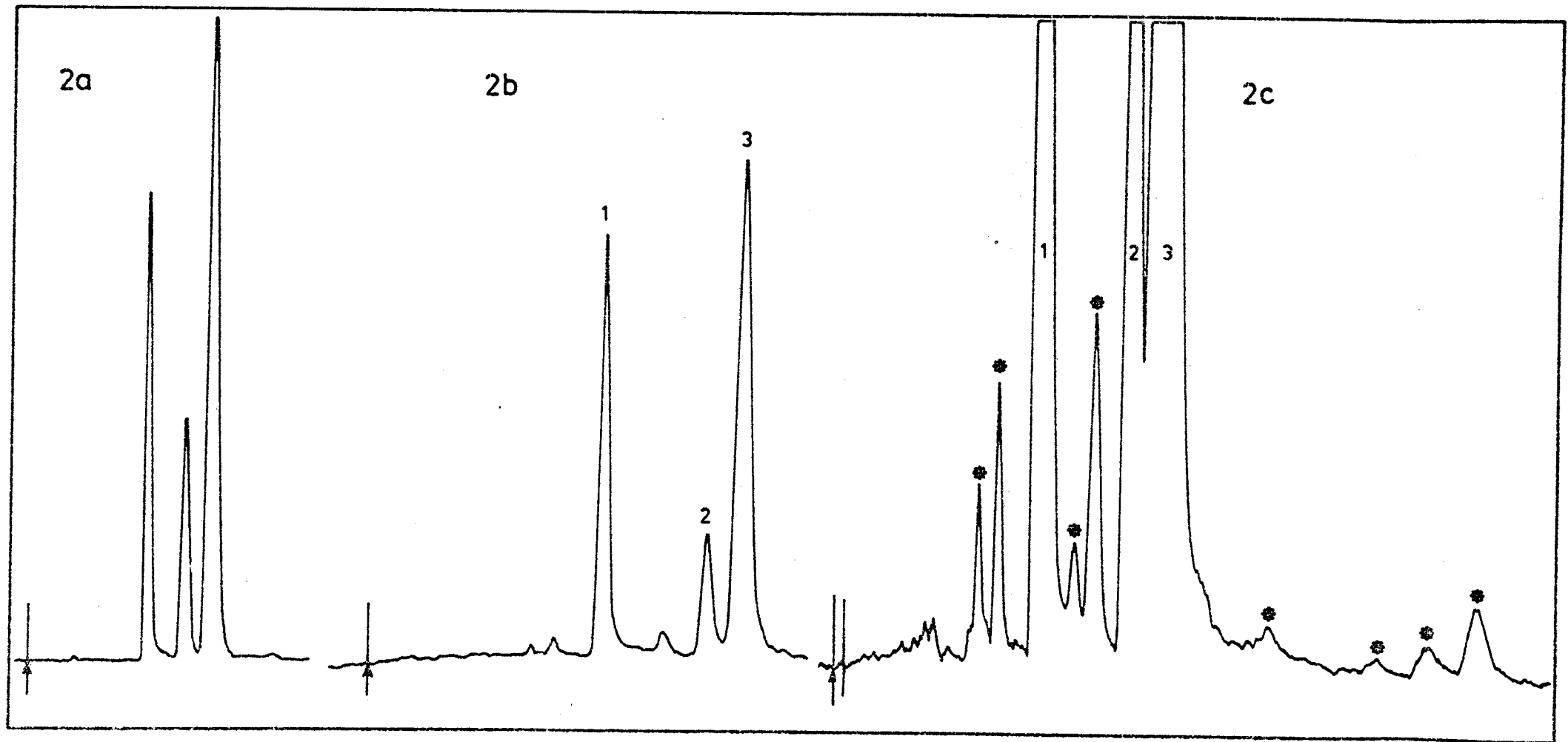
13. Checking the HPLC instrument by establishing the calibration equation is essential. This may or may not be the same as the one described in this paper. To establish this calibration curve a hop extract with known composition is available from our laboratory. We used three different instruments; there were differences. The data in the paper are as example and were obtained with the set up most used in the study.
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Legend to figures

Fig. 1 : HPLC trace of hop extract. Column : 25 x 0.46 cm, RSil-C₁₈ (17 %) 10 μ acid washed. Varian 8520 Liquid Chromatograph, Valco 7000 psi 10 μ l loop injection. Varichrom detector at 314 nm. Eluting solvent : methanol (85 ml), water (17 ml), phosphoric acid 85 % (0.25 ml), BHT (1 g). Full chromatographic details in paper. Peaks in order of appearance : 1 : unknown, 2 : chalkone internal standard, 3 : cohumulone, 4 : humulone-adhumulone, 5 : colupulone, 6 : lupulone.

Fig. 2 : A : Purified lead salt of α -acids; conditions as in Fig. 1. Peaks for chalkone, cohumulone and humulone-adhumulone.
B : Purified lead salt of α -acids; conditions as in Fig. 1 but without internal standard and with eluting solvent : methanol (59 ml) 0.2 M acetate buffer pH 7.0 (41 ml). Major peaks in order of appearance : 1 : cohumulone, 2 : adhumulone and 3 : humulone.
C : Idem as Fig. 2B but with larger sample size. Extra peaks (x) integrate for 1 % of total area.







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Zoeterwoude, January 1980



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APPENDIX I

Minutes of the 50th Meeting of the EBC Analysis Committee, Bruges,
11th December 1979

REPORTS OF SUBCOMMITTEES

a. ANALYTICA MICROBIOLOGICA (Chairman: H.B. Pfenninger, CH)

Present: H.B. Pfenninger (Chairman), M. Benard, D. Eyben, P.A. Martin, M. Moll, L. Narziss, G.H. Ulenberg and Mrs. M. van Wijngaarden.

Because of the promised assistance of Mr. Martin, Messrs. Pfenninger and Moll withdrew their request for replacement in the Subcommittee. In the meantime, Mr. Martin drafted three methods:

2.4.2 : Lactobacillae

2.4.3 : Zymomonas

2.5.2 : Introduction to Flocculation

Messrs. Leedham and Niefind would also co-operate; Mr. Leedham would draft the method on *Pediococcus* (2.4.6), but so far nothing has been received. From Mr. Niefind nothing at all has been heard. Dr. Pfenninger would contact him once more; should this not be successful, then Mr. Moll would approach M. Germain (F).

Drafting of the following methods is concerned:

2.3.1.1 on cell forms

2.3.1.2 on colony forms

2.3.2.4 on selective media

Mr. Martin agreed to draft the tests on sedimentation and flocculation.

It was decided to continue publication in three languages, especially for the benefit of laboratory workers. It was agreed that translations would be made as follows:

<u>by</u>	<u>from</u>	<u>into</u>
Pfenninger	English	German
Pfenninger	French	German
Martin (assisted by Dr. MacWilliam)	French	English
Martin (assisted by Dr. MacWilliam)	German	English
Moll	German	French
Benard	English	French

This set-up resulted in translation of the following methods by:

- Pfenninger (into German)
2.2.2.2 - 2.3.2 - 2.3.2.3 - 2.3.2.6 - 2.3.2.7 - 2.4.1.1 - 2.4.1.2 -
2.4.5 - 2.5.1 - 2.5.5

- Martin (into English)
2.2.2.2 - 2.3.2.1 - 2.3.2.2 - 2.3.2.3 - 2.4.1.1 - 2.4.1.2 - 2.4.5 -
2.5.1 - 2.5.5



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- Moll (into French)
2.3.2.2
- Benard (into French)
2.3.2.6 - 2.3.2.7

The above-mentioned method Nos. 2.4.2, 2.4.3, and 2.5.2, drafted by Mr. Martin, first would be commented on by the members of the Subcommittee, in writing. The final set would be sent to the Secretariat of EBC, whereupon the Secretariat would send copies to Dr. Pfenninger and Mr. Benard for translation.

It was agreed that publication of *Analytica Microbiologica* part II should be organised through the three brewing journals that also published part I: *Brauwissenschaft*, *BIOS* and the *Journal of the Institute of Brewing*, but that no reprints would be ordered. Those who are interested may be supposed to read at least one of these journals.

Because publication through the *Journal* would take at least 9-12 months, it was agreed that the Secretariat would already inform the editors.

Dr. Pfenninger announced that he would like to be discharged as Chairman of this Subcommittee at the next meeting of the Main Committee of the Analysis Committee.

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b. DIACETYL (Chairman: P. Gjertsen, DK)

Mr. M. Sopo has joined the Diacetyl Subcommittee.

The Subcommittee now concentrates its work on the gaschromatographic determination of vicinal diketones and their precursors. Methods have been collected from the members, including K.J. Siebert, ASBC, and after comparison of these methods a proposal for a method to be tested was set up.

After circulation among the members the proposal was discussed at the SC-meeting, and the participants have agreed upon a description of the method.

Two pairs of beer samples will be sent to the members for analysis, one pair with low diacetyl content and another pair with higher content. The results will be discussed before the next meeting.

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c. ENZYMIC ACTIVITIES (Chairman: S. Lie, N)

Since the last meeting in Berlin new tablets for the alpha-amylase test have been prepared by Pharmacia. These tablets were tested in the Main Committee, comparing them with four malt samples and the 9th EBC-standard malt. The results obtained showed a coefficient of variation around 30% for the four malt samples and 10% for the EBC-standard malt. Among the members of the Subcommittee who are trained in carrying out this method, the coefficient of variation was reduced to a level of 20%. A correction of the results by means of the standard malt improved the variation to some degree.

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The Subcommittee judged the results obtained as not quite satisfactory and recommended that further improvements to the method should be tried out. The main improvement suggested was to introduce stirring during the reaction period, the same type of stirring which improved the endopeptidase method. Further modifications of the details of the present draft alpha-amylase method were also introduced.

The modified method will be tested again in the Subcommittee. It is hoped that the modifications introduced will reduce the coefficient of variation to 10-15%, which seems to be the limit for the accuracy of this type of method.

For the endopeptidase method it was reported that the Calbiochem-Behring Corp. are working on the production of Hide Powder Azur tablets, but that the tablets are not yet ready. The producer of the tablets will be contacted and asked to incorporate cysteine in the tablets but no buffer.

A preliminary test with a draft beta-glucanase method was also discussed. Substrates from both NOVO and BIOCON had been tested and it was decided to ask both companies to produce a substrate according to specifications laid down after further contacts with specialists in this field.

It was decided that lichenan was not suitable as substrate since it is no longer available in a pure form.

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d. EXTRACT OF ADJUNCTS (Chairman: I. Rosendal, DK)

A collaborative test has been made for maize starch, maize grits, rice and barley, and the results have been distributed among the members of the Subcommittee. At the meeting these results were given to the members of the Main Committee. Dr. Pfenninger pointed out an error made in the input of one of his results.

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A corrected page is enclosed but the correction does not change the conclusion. (See Appendix II)

Although the collaborative test has shown better results for the Termamyl method compared with the standardized EBC or INT method, the Subcommittee suggested only to standardize the Termamyl method for maize (starch and grits), because it is known that special varieties of rice can give problems and because of the observed prolonged filtration time for barley. Moreover, rice is not so much used in Europe.

The Main Committee accepted that Mr. Rosendal will make a draft for this new method, to be circulated before the next meeting. Members were requested to send their comments to Mr. Rosendal direct, to be discussed in the May-meeting of 1980, after which the method will be finalized, including mention of the restrictions. Also Mr. Frohmader (ASBC) will be kept informed.

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e. FILTER AIDS (Chairman: D. Eyben, B)

1. Reference method for permeability determination

The EBC Analysis Committee has given its permission for publication in the brewing press of a reference method for the determina-

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tion of the permeability of filter aids, expressed in DARCY units.

This determination can be made with the "Perméamètre" made by CECA Ltd., 11 Avenue Morane Saulnier, F-78140 Veliny-Villacoublay (France).

Any other apparatus adapted to the specification of this method can be used as well.

2. Routine method for filtration speed determination

A simplified apparatus for the determination of filterability is being constructed and will be tested by the Subcommittee.

3. Control of filter sheets

The Subcommittee now proposes to start studying control and analysis methods for filter sheets. All suggestions in this field can be passed on to Mr. D. Eyben, Chairman of the Subcommittee, or to the EBC Secretariat, which will forward them.

Through the Belgian section, the International Filtration Society ("Société Internationale de Filtration") will be contacted.

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f. FLAVOUR (Chairman: M. Moll, F)

The Subcommittee did not meet this time.

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g. GERMINATION (Chairman: W.J. Klopper, NL)

With dormant or damaged barleys higher values for the Aubry-tests were found than with the Schönfeld-tests. Maltsters in the Netherlands consider the Schönfeld-test the better one to give an impression of the behaviour of a barley in the maltings. The barley trade, on the other hand, prefers the Aubry method, as this may yield higher results. Hence, there is reason for action by EBC to clarify the position.

Prof. Narziss can confirm these findings. He found 5-8% higher Aubry values with dormant and damaged barleys and considers this matter important.

Mr. Moll will ask M. Deymié to send relevant information to Mr. Klopper.

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h. HOPS AND HOP EXTRACTS (Chairman: J.R. Hudson, GB)

Present: Messrs. G. Ganzlin, J.R. Hudson (Chairman), M. Moll, H.B. Pfenninger, I. Rosendal; J. Maier had apologised for his absence.

1. The Chairman reported that neither the Subcommittee nor the Main Committee had requested any changes in the paper justifying the recommendation of the Sephadex methods. Hence, the paper had been sent to Mr. Ulenberg for publication in all journals.

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2. No changes had been asked for in the draft revisions for Analytica, and these have also been passed to Mr. Ulenberg. However, it was noted that there is an error on p. 3 of 6.3.4, where the temperature for pre-mixing the sample should be 40° and not 60°.
3. The analysis of the new standard extract was considered satisfactory and hence the Secretariat will be asked to hold the samples and fix the charges. Mr. Ganzlin said that Lupofresh would supply the extract free to EBC, and the Subcommittee expressed gratitude for that gift.
4. It had not been possible to find a suitable isomerised extract to serve as analytical standard. It was decided to wait until products from the liquid carbon dioxide processes are available to make a fresh attempt.
5. The results of the comparison of the modified Verzele conductometric method with the existing Analytica method showed no improvement in agreement between laboratories. The results were, however, higher and from MEBAK trials, closer to the results from the Ganzlin method. It was decided to make no change in Analytica at the present time because of the failure to obtain better agreement between laboratories.
6. It was agreed that members would exchange methods for HPLC analysis, but that there will be no formal collaborative trial of these methods at present.
7. Increasing concern was noted about the safety of solvents used in hop analysis. Several solvents are suspected of being teratogens. Hexane is apparently the only safe solvent, but it is ineffective in treating stored hops. HPLC methods might circumvent this difficulty.
8. Following the resignation of Dr. Hudson, the Subcommittee recommended that Mr. I. Rosendal be appointed Chairman. Dr. Hudson expressed thanks to all who had assisted the Subcommittee in the past.

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i. INSTRUMENTAL COLOUR MEASUREMENT (Chairman: B.W. Drost, NL)

The results of two test series of measurements were discussed:

1. A collaborative test of two samples, according to the EBC disc method and the proposed photometric method. Results were received from 18 members. Only three members were in the possession of a photometer provided with the recommended interference filter. In spite of that, the F-value for the EBC method is much higher than for the absorption method.
2. Individual tests for each collaborator (about 100 samples); both own worts and beers were measured in both ways, so the results of the different laboratories could be compared. The results were divided into 4 series and the coefficients of variation were calculated. In three out of the four cases, the mean value of the EBC method was higher than the absorption method value.

Future work: Five samples of beer will be sent to all members who want to co-operate. Both methods will have to be used again. However, for the instrumental method no special instrument will be prescribed.

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Only, every member has to set the wavelength exactly and frequently, and is requested to indicate his wavelength setting method together with his results.

A working method will be sent with the samples.

After some discussion, Dr. Drost was requested to draft a press report, stating that the instrumental method is recommended as reference method and that the EBC-method is less reliable, due to the unreliability of the colour discs. The Chairman asked the Subcommittee to continue its work.

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i. MALT ANALYSIS (Chairman: M. Moll, F)

1. DLFU Bühler-Miag Mill

M. Moll will write to Bühler-Miag to insist that the mill be calibrated in the way described in Analytica III. Bühler-Miag will be asked whether it is possible to supply a maintenance contract service.

2. Tepral method

The Subcommittee was unable to carry out the method in the way described in the note of 26-07-1979. It was decided that all the participants would be equipped with the vacuum device by 01-02-1980. A new series of two malts will be sent for a new interlaboratory test.

It was stated that the Buchner should be modified in order that the filter bed could be cleaned.

Dr. Klopper invited the members to visit the new, computer-controlled, research brewhouse of NIBEM/TNO, during a next meeting at Zeist.

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k. OXALATE (Chairman: P. Gjertsen, DK)

As mentioned in the preceding report of the Subcommittee, two methods for the determination of oxalate in beer would be investigated.

Preliminary work has been performed by a few members. The results were discussed at our meeting, and it was decided that both methods will be tested by the Subcommittee.

The beer samples used in the preliminary work had low oxalate contents, because they had been produced with hard water. The Chairman will prepare two pairs of beer samples for the analyses, one pair with low oxalate content, and another pair with higher content, produced in the pilot brewery with very soft water.

On the basis of the results of the analyses it will be discussed at the next meeting whether one of the two methods is satisfactory. The amount of manual work, the total time required for an analysis and the costs, will also be compared.

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1. SAMPLING OF RAW MATERIALS (Chairman: I. Rosendal, DK)

The promised comments by ASBC have still not been received and it is not possible for the Subcommittee to continue its work any further.

M. Moll promised to write ASBC (M. Meilgaard) and to ask for a quick answer.

As soon as the final results on hop sampling would be known, Dr. Pfenninger would send them to Mr. Rosendal.

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m. SUGARS AND CARBOHYDRATES IN BEER AND WORT (Chairman: P.A. Martin, GB)

1. Total carbohydrate in beer

Members had collaboratively tested the Institute of Brewing anthrone method and the Drawert and Hagen enzymatic method. Special care was required with the Drawert and Hagen method to ensure complete hydrolysis of the carbohydrate.

The Subcommittee recommended that the Analysis Committee test and adopt the Institute of Brewing method.

The Subcommittee would also ask the Analysis Committee whether they wished to consider recommending a method for determining the caloric value of beer based upon the total carbohydrate, alcohol and protein contents.

2. HPLC of carbohydrates in wort

Members were still involved in commissioning new equipment and methods in their laboratories. It was agreed that no new collaborative work would be planned until the results of the current ASBC collaborative trial, using an Aminex HPX 87 column, had been seen.

Mr. Moll proposed to introduce caloric value in Analytica. Since there are different regulations in the various European countries, it was decided that all members should send the regulations effective in their country to Mr. Martin, so that he would be able to make a comparison. A report would be given during the next meeting. Mr. Martin would also inform ASBC.

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n. CO₂ DETERMINATION (Chairman: R. Westelius, S)

A first set of collaborative results has been obtained, with a simple manometric method, showing excellent reproductivity, with a fair and promising agreement between laboratories. A revised description of the method will be sent, together with new sets of samples, for a further series of collaborative study.

It was emphasised that EBC needed official CO₂ analysis methods.

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THE BREWING RESEARCH FOUNDATION

Research Establishment

From J.R. HUDSON, Ph.D., F.R.I.C.
Deputy Director

Lyttel Hall, Nutfield,
Redhill, Surrey.
RH1 4HY

Tel: Nutfield Ridge (073 782) 2272
Telex: 8814000

24th September, 1979

Dear Gerald,

At last I enclose the revised and new methods for the Hops Section of ANALYTICA.

Will you please arrange the printing at the appropriate time. The first page, headed 'Brewing Research Foundation' is simply for your guidance as to what the contents of this package are and will not, of course, appear in ANALYTICA.

With kind regards,

Sincerely yours,

P.S. I should much appreciate it if I could have the English language proofs for checking.

Ing.G.H.Ulenberg,
Secretary-General of the EBC,
P.O.Box 510,
2380 BB ZOETERWOUDE,
The Netherlands.

September 1979.

Brewing Research Foundation.

New or Revised Methods for Analytica - EBC

- 6.2.1 Determination of the seed content of hops (International method). This is a new method.
- 6.3. Alpha Acids and related Substances in hops, hop powders and hop extracts. - This revised introduction replaces the section in Analytica and includes a section on safety.
 - 6.3.1. The conductometric lead value for hops and hop powders. Add footnote to the method in Analytica.
 - 6.3.2. Bitter substances in hops, hop powder and hop extract powder. This is a new method which replaces existing method 6.3.2. in Analytica.
 - 6.3.2.1. Bitter substances in hop extracts. - This is a new method for Analytica.
 - 6.3.4. Determination of specific hop compounds and their derivatives (International method) - This is a new method for Analytica.
 - 6.3.5. Determination of α -acids in hops, hop powders, hop pellets and hop extracts (International method) - This is a new method for Analytica.
 - 6.3.6. Estimation of iso- α -acids in isomerized hop extracts (International method) - This is a new method for Analytica.

6.2.1 DETERMINATION OF THE SEED CONTENT OF HOPS
(International Method)

Scope

The method can be applied to samples of hop cones.

Principle of Method

The hops are heated to reduce the sticky properties of the resins. The seeds are dislodged from the hop cones, separated from strig and petal and weighed.

Apparatus

Suitable sieve.

Metal container with a loose-fitting lid.

Oven. A hot-air oven regulated to give a temperature of 115°C.

Procedure

500 g of the sample is placed in a metal container with a lid and heated for two hours at 115°C to remove the sticky properties of the resins. The dried sample is wrapped in coarse cotton cloth and rubbed vigorously or beaten mechanically, in order to detach the seeds from the hops. A sieve is used to separate the dried and finely fragmented hops from the seeds. Any stems or petals remaining with the seeds are separated using either a sloping surface covered with emery paper or any other method which gives the same result, e.g. by holding the stems and other matter and permitting the seeds to roll off. The seeds are then weighed.

Expression of Results

The seed content is calculated as the percentage of seeds in the original sample.

References

Methods of Analysis of the American Society of Brewing Chemists,
7th Edition, 1976.

U.S. Department of Agriculture, Inspection Handbook for Hops,
USDA, AMS, Grain Division, Rm 0642, 1400 Independence Ave., S.W.,
Washington, D.C., 20250. (Revised June 1976).

Monroe, C.G. and Hill, D.D., J. Amer. Soc. Agronomy, 1939,
31, 698 - 700.

6.3. Alpha Acids and Related Substances in Hops, Hop Powders, Hop Extracts and Isomerised Hop Extracts.

The bittering capacity of freshly harvested hops is almost entirely due to the α -acids. This is also true of hop-powders and extracts made from them, provided that the manufacturing processes are not defective. However, when hops have been stored the α -acids contents decline, mainly due to oxidation, at rates which depend on storage conditions and on hop-variety. Some oxidation products of both α -acids and β -acids are bitter so that the bittering capacities of stored hops and of powders and extracts made from them are greater than those indicated by the α -acids contents. Many of the oxidation products react with lead acetate and experience has shown that the "Conductometric Lead Value" gives a more reliable indication of bittering capacity than do the specific methods. However, the different groups of compounds have different utilisation factors and utilisations also vary from brewery to brewery so that it is necessary to establish empirical correlations between Conductometric Lead Values and bitterness for individual breweries.

In Central Europe great importance is attached to measurement of the total resin, total soft resin and hard resin so a modification of the Wöllmer method is given which also affords a measure for the Conductometric Lead Value.

The bittering capacity of isomerised hop extracts lies solely in their content of iso- α -acids and specific analysis can be applied. The presence of α -acids or β -acids in isomerised hop-extracts diminishes utilisation of the iso- α -acids. Hence chromatographic methods are necessary to ascertain the purity of such products and to monitor efficiency in their production. Chromatographic methods are also necessary for the measurement of specific groups of compounds in hops, hop products and hop extracts.

It must be emphasised that most of the methods used to analyse hops, hop products and beer for bitter substances are empirical, i.e. the value obtained depends on the conditions used. It is therefore essential that the method of measurement is stipulated in contracts and specifications and when results are reported.

Checking the Methods

It is sound practice to check the methods periodically by analysing reference standards. For this purpose EBC holds a standard hop extract. This, together with the established analyses, can be obtained from:

The EBC Secretariat, P.O.B. 455, Rotterdam, Holland.

Safety Notes

Diethyl ether, hexane, benzene, toluene and methanol are all highly flammable and vapour-air mixtures can explode on ignition. Do not use these solvents in the presence of naked flames. Always work in a fume cupboard when using benzene. There is a risk of poisoning if methanol is inhaled or taken internally. Avoid contact with the skin and eyes when using benzene, methylene chloride or dimethyl sulphoxide.

Concentrated hydrochloric, sulphuric and acetic acids are corrosive and can cause burns. Acid fumes are also irritating to the respiratory system. If these acids are in contact with the eyes rinse immediately with water and seek medical advice.

Lead acetate solution is harmful if taken internally and, as with all lead salts, there is a danger of cumulative poisoning.

Rotary evaporators and other apparatus operating under vacuum should be shielded by safety screens.

After centrifugation the lid of the centrifuge should not be raised until the rotor has come to rest.

Do not pipette any solutions by mouth.

6.3.1. The Conductometric Lead Value for Hops and Hop Powders.

Add the following footnote to the method in Analytica

Footnote:

Use 2% lead acetate solution for traditional varieties of hops of 3 - 5% α -acid and 4% lead acetate solution for hops containing more than 6% α -acid.

6.3.2. Bitter Substances in Hops, Hop Powder and Hop Extract Powder

(Ganzlin modification of Wollmer method)

From the brewing point of view these are considered to be the most important constituents of hops. They comprise a whole series of chemical compounds with different properties. The analytical values obtained depend on the product and the method used.

Principle

The hop bitter substances are distributed between an acidic-aqueous-methanolic phase and diethyl ether. The bitter substances extracted by ether are subsequently fractionated according to their varying solubility in cold methanol and hexane as total, soft and hard resin. The α -acids are separated from the β -fraction in the soft resin by utilizing the ability of α -acids to form lead salts, (Conductometric Value).

Reagents

Hydrochloric acid solution, 0.1N.

Diethyl ether, max. 0.2% water, peroxide free. (examined with test strips).

Cotton wool roll.

Methanol, analytical grade.

Methylene chloride, analytical grade.

n-Hexane, analytical grade (purity based on the residue after distillation and gas chromatographic examination).

Lead acetate, 2% . 20 g Pb (C₂H₃O₂)₂ · 3H₂O dissolved in 1 litre methanol containing 0.5 ml acetic acid or in ethylene glycol monoethyl ether.

(Obtainable as Titrisol Merck Cat. No.9897).

Lead acetate solution, 4% . 40 g Pb (C₂H₃O₂)₂ · 3H₂O dissolved in 1 litre methanol containing 0.5 ml acetic acid, or in ethylene glycol monoethyl ether.

Use 2% lead acetate solution for traditional varieties of hops of 3-5% α -acid and 4% lead acetate solution for hops containing more than 6% α -acid.

Before use, the lead acetate concentration is measured by conductometric

titration against 2.0 ml of 0.1N sulphuric acid in 40 ml of dimethylsulphoxide-ethanol mixture.

$$\% \text{ Lead acetate trihydrate} = \frac{1.897 \times 2}{\text{ml lead acetate solution used}}$$

Sulphuric acid solution, 0.1N.

Ethanol, 96%.

Dimethylsulphoxide, analytical grade.

Dimethylsulphoxide-ethanol mixture, mix 3 volumes dimethylsulphoxide with 47 volumes ethanol.

Titriplex III or Complexon III (e.g. Merck No.8421 or Riedel de Haen No.34549).

Sodium hydroxide solution, 0.2N.

Electrode cleaning solution: 2.0 g Complexon III in 100 ml sodium hydroxide solution.

Apparatus

Glass flasks with solvent-tight screw-stoppers - 250 ml (e.g. Sovirel or Schott).

Wide-mouthed glass funnel of corresponding size.

Glass centrifuge tubes with solvent-tight screw caps or ground glass stoppers (100-110 ml capacity) (e.g. order from Glasbläserei Hauck, Pirckheimer Str. 51, D-85 Nürnberg).

Analytical balance, accuracy ± 0.5 mg.

Pipettes, 2 ml, 10 ml, 30 ml, 40 ml, 50 ml, 100 ml.

Pipette ball.

Dispenser, 5 ml, 10 ml, 20 ml, 25 ml, 40 ml.

Automatic shaker.

Angled Centrifuge

Flat-bottomed flask with NS 29/32 joint, 100 ml.

Water-bath, 20°C.

Rotary vaporator with water-bath at 70°C.

Wash-bottle, stable to solvent.

Cylinder containing carbon dioxide or nitrogen, pressure reduced to 0.2 bar.

Graduated flask, 50 ml. Glass funnel and suitable clock-glass, (diameter approx. 80 mm).

Cooling bath at 0°C.

Fluted filter paper, diameter 12.5 cm.

Vacuum adaptor with NS 29/32 joint and stopcock to gas-inlet tube.

Desiccator with drying agent (silica gel with indicator).

Wide-necked Erlenmeyer flask, 50 ml, or

Tall glass beaker, 100 ml.

Automatic apparatus for conductometric titration, (e.g. Konduktoskop) or

Conductivity meter with immersion electrode, magnetic stirrer and microburette with 0.01 ml divisions. The electrodes must consist of bright platinum.

If they are protected by glass covers, these must have suitable openings so that the air can escape and the reaction solution can flow freely round the whole surface of the electrode.

Clearing of Electrodes

After use, electrodes are kept in the cleaning solution. The latter is prepared weekly.

Procedure

Preparation of Stock Solution About 10 g of finely ground hops or hop powder are transferred to a tared glass flask and weighed accurately. 20 ml of methanol and 100 ml of ether (20°C) are added by pipette. The glass flask is stoppered carefully and attached to a shaker. After shaking the flask for 30 minutes, it is opened carefully and 40 ml of 0.1N hydrochloric acid added. The flask is stoppered carefully and shaken for a further 10 minutes at the optimal speed. It is then removed from the shaker and allowed to stand for 10 minutes. Cotton wool is twisted slightly around the tip of a 50 ml pipette and a pipette ball attached. The ether phase is drawn into the

pipette to a level of 6 cm above the calibration mark. In cases where there is too little supernatant only 40 ml is taken, but this has to be allowed for in the calculation. The pipette is removed from the flask and at the same time the cotton wool attached to the pipette is scraped off on the neck of the flask. The volume of supernatant in the pipette is adjusted to 50 ml, transferred to a flat-bottomed flask and 25 ml of methylene chloride added. The solvent is removed by rotary evaporation at 70°C. About 5 ml of methanol is added by wash-bottle through the gas inlet tube that projects into the flat-bottomed flask. The angle of inclination of the flask is reduced slightly and the flask rotated for a further period until the extract adhering to the flask wall has dissolved completely. The flask is flushed with carbon dioxide or nitrogen, removed from the rotary evaporator and a further 5-10 ml methanol added. The methanolic extract solution is transferred quantitatively through a glass funnel to a 50 ml graduated flask using methanol to rinse the flat-bottomed flask and funnel. The solution is mixed by gentle shaking, attemperated to 20°C and made up to the mark with methanol. The graduated flask is stoppered and allowed to stand for 1 hour at 0°C, to precipitate the hop waxes. A filter funnel, fitted with a dry fluted filter paper, is placed in the neck of a 50 ml graduated flask. The cold methanolic extract solution is poured without stopping into the fluted filter paper. During the filtration the funnel is covered with a clock glass. The filtrate in the graduated flask (not made up to volume) is attemperated to 20°C. This is the stock solution.

Determination of Total Resin 10.0 ml of the stock solution is pipetted into a clean, dry (kept for at least 30 minutes in a desiccator) and tared 100 ml flat-bottomed flask (neck not greased). The methanol is distilled off using a rotary evaporator, at about 70°C under vacuum. When the residue in the flask is dry it is removed from the rotary evaporator and attached to a

vacuum adaptor. The residue in the flask is dried at 70°C for 6 minutes under a high vacuum (less than 15 Torr). The vacuum is released and using the water pump, air is briefly drawn through the flask. The flask is dried carefully with a soft cloth, then kept for 30 minutes in a desiccator and weighed.

Calculation

$$\text{Total Resin \% w/w} = \frac{\text{Weight of residue (g)} \times 1000}{\text{Initial weight (g)}}$$

The results are given to one place of decimals.

Accuracy = ± 2% (Coefficient of Variation).

Determination of Soft Resin 10.0 ml of methanolic stock solution, 5 ml of methanol (preferably by dispenser) and 40.0 ml of n-hexane are mixed together in a glass centrifuge tube. 10.0 ml of 0.1N hydrochloric acid is added (preferably by dispenser). The centrifuge tube is stoppered and shaken vigorously for about 30 seconds by hand or for 5 minutes using a shaker, after which it is centrifuged for 2 minutes at 2000 rpm to separate the phases. 30 ml of the clear upper hexane phase is pipetted into a clean, dry (kept for at least 30 minutes in a desiccator), tared flat-bottomed flask (neck not greased). Using a rotary evaporator, the hexane is distilled off under slight vacuum at 70°C. The flask is removed from the rotary evaporator and attached to a vacuum adaptor. The residue in the flask is dried at 70°C for 6 minutes under a high vacuum (less than 15 Torr). The vacuum is slowly released and using the water-pump air is drawn briefly through the flask. The flask is dried carefully with a soft cloth, then kept for 30 minutes in the desiccator and weighed.

Calculation

$$\text{Soft Resin \% w/w} = \frac{\text{Weight of residue (g)} \times 1333}{\text{Initial weight (g)}}$$

The results are given to one place of decimals.

Accuracy = $\pm 3\%$ (Coefficient of Variation).

Determination of Conductometric Value 10 ml of stock solution is pipetted into a wide-necked Erlenmeyer flask or tall beaker followed by 40 ml of dimethylsulphoxide-ethanol mixture. A stirring bar is added and the flask or beaker stirred using a magnetic stirrer. The measuring cell of the automatic titrator is immersed to the correct depth in the liquid. It may be necessary to add more dimethylsulphoxide-ethanol mixture. The burette containing lead acetate solution is placed so that the fine tip is immersed 3 to 4 cm into the test solution. The automatic titrator is switched on and the plot recorded. The intersection of the straight lines for salt precipitation and addition of excess lead acetate solution is the equivalence point. In order to obtain a significant number of points on the straight line for the addition of lead acetate solution, the titration is continued beyond the equivalence point.

Manual Titration The lead acetate solution is added in 0.25 ml portions and the conductivity recorded after each addition. The titration is continued past the point of inflection until a straight line has been obtained. A graph of conductivity against ml of lead acetate solution is plotted. From the graph the volume of lead acetate solution used for precipitation is employed to calculate the Conductometric Value.

Calculation

$$\text{Conductometric Value \% w/w} = \frac{\% \text{ lead acetate} \times T \times 9.43}{\text{Initial weight (g)}}$$

where T = Titre of lead acetate solution

The results are given to one place of decimals.

Accuracy = $\pm 5\%$ (Coefficient of Variation).

Determination of β -Fraction

Calculation

β -Fraction % w/w = Soft Resin - Conductometric Value

The results are given to one place of decimals.

Determination of Hard Resin

Calculation

Hard Resin w/w = Total Resin - Soft Resin

The results are given to one place of decimals.

Normal Values

	Whole Hops Hop Powder	Enriched Hop Powder	Hop Extract Powder
	% as is	% as is	% as is
Total Resin	12-20	22-40	30-60
Soft Resin	10-18	18-36	24-54
Conductometric Value	4-11	7-20	9-30
β -Fraction	6-9	11-16	15-24
Hard Resin	2-4	2-7	3-10

Evaluation

Reporting of the Conductometric Values and β -Fraction as percentages of the total resin allows certain conclusions to be drawn on the variety and growing region of the raw or processed hops. In addition, the ratio of percentages of hard resin to total resin allows an evaluation of the degree of ageing.

Generally, hops with a hard resin content of above 13% can be considered as aged. A hard resin content of over 17% in hop powders is also viewed as too high. It must be considered, when interpreting the results, that the determination of hard resin content has a coefficient of variation of at least $\pm 10\%$.

Note

During the whole of the analysis, the effects of light, oxygen and heat should be minimised.

References

Pfenninger, H., Schur, F. and Senn, R. Schweiz. Brauerei-Rdsch. 1973, 84, 183-190.

Ganzlin, G., Brauwissenschaft, 1975, 28, 231-239.

Ganzlin, G., Brauwissenschaft, 1975, 28, 344-346.

6.3.2.1. Bitter Substances in Hop Extracts (Ganzlin modification of Wöllmer method)

The composition of bitter substances in hop extracts corresponds normally to that of the original hops.

Principle

The hop extract constituents are distributed between an acidic methanolic phase and diethyl ether. The bitter substances extracted by ether are subsequently fractionated according to their varying solubility in cold methanol and hexane as, total soft and hard resin. The α -acids are separated from the β -fraction in soft resin by the ability of α -acids to form lead salts (Conductometric Value)

Reagents

Hydrochloric acid solution, 0.1N.

Diethyl ether, max. 0.2% water, peroxide free (examined with test strips).

Methanol, analytical grade.

Methylene chloride, analytical grade.

n-Hexane, analytical grade (purity based on residue after distillation and gas chromatographic examination).

Lead acetate solution, 2% . 20 g $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$ dissolved in 1 litre methanol containing 0.5 ml acetic acid or in ethylene glycol monoethyl ether.

(Obtainable at Titrisol Merck Cat. No.9897)

Lead acetate solution, 4% . 40g $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$ dissolved in 1 litre methanol containing 0.5 ml acetic acid, or in ethylene glycol monoethyl ether.

Use 2% lead acetate solution for standard extracts and 4% lead acetate solution pure resin extracts.

Before use, the lead acetate concentration is measured by conductometric titration against 2.0 ml of 0.1N sulphuric acid in 40 ml dimethylsulphoxide-ethanol mixture.

$$\% \text{ Lead acetate trihydrate} = \frac{1.897 \times 2}{\text{ml of lead acetate solution used}}$$

Sulphuric acid solution, 0.1N.

Ethanol, 96%.

Dimethylsulphoxide, analytical grade.

Dimethylsulphoxide-ethanol mixture: Mix 3 volumes dimethylsulphoxide with 47 volumes ethanol.

Titriplex III or Complexon III (e.g. Merck No. 8421 or Riedel de Haen No. 34549).

Sodium hydroxide solution, 0.2N.

Electrode cleaning solution: 2.0 g Complexon III in 100 ml sodium hydroxide solution.

Apparatus

Glass centrifuge tubes with solvent-tight screw or ground-glass stopper, 100-110 ml capacity (e.g. order via Glasbläserei Hauck, Pirckheimer Str. 51, D - 85 Nurnberg).

Analytical balance, accuracy ± 0.5 mg.

Pipettes 2 ml, 10 ml, 30 ml, 40 ml, 50 ml.

Pipette ball.

Dispenser, 5 ml, 10 ml, 20 ml.

Automatic shaker.

Angled Centrifuge.

Flat-bottomed flask with NS 29/32 joint, 100 ml.

Water-bath 20°C.

Rotary evaporator with water-bath at 70°C.

Wash-bottle, stable to solvent.

Cylinder of carbon dioxide or nitrogen, (pressure reduced to 0.2 bar).

Graduated flask, 50 ml, glass funnel and suitable clock-glass, (diameter approx. 80 mm).

Cooling bath, 0°C.

Fluted filter paper, diameter 12.5 cm.

Vacuum adapter with NS 29/32 joint and stopcock to gas-inlet tube.

Desiccator with drying agent (silica gel with indicator).

Wide-necked Erlenmeyer flask 50 ml, or tall glass beaker, 100 ml.

Automatic apparatus for conductometric titration, (e.g. Konduktoskop) or

Conductivity meter with immersion electrode, magnetic stirrer and micro-

burette, graduated in 0.01 ml divisions. The electrodes must consist of bright platinum. If they are protected by glass covers, these must have suitable openings so that air can escape and the reaction solution can flow freely round the whole surface of the electrode.

Cleaning of Electrodes.

After use electrodes are kept in the cleaning solution. The latter is prepared weekly.

Procedure

Preparation of Stock Solution A sample corresponding to about 0.6 g -- 0.7 g total resin is thinly streaked using a spatula on the inside of a stoppered, tared glass centrifuge tube and weighed accurately to 1 mg. 20 ml of 0.1N hydrochloric acid and 50 ml of ether are pipetted into the tube. The centrifuge tube is tightly stoppered and either shaken for 5 minutes using an automatic shaker or manually, until the whole of the extract on the glass wall has dissolved. The centrifuge tube is carefully opened and 10 ml of methanol added. The centrifuge tube is carefully stoppered and shaken for at least a further 15 minutes at optimal speed on the shaker, after which it is centrifuged for 2 min. at 2000 rpm. 40 ml of the clear ether phase is pipetted into a flat-bottomed flask and 20 ml of methylene chloride added. The solvent is evaporated using a rotary evaporator on a water-bath at 70°C. As soon as the extract solution is removed, 5 ml of methanol is added from a wash bottle through the gas inlet tube that projects into the flat-bottomed flask. The angle of inclination of the flat-bottomed flask is reduced and the flask rotated for a further period until the extract adhering to the flask wall has dissolved completely. A glass funnel is placed in a 50 ml graduated flask. The flat-bottomed flask is flushed with carbon dioxide or nitrogen and removed from the rotary evaporator. A further 5 - 10 ml of methanol is added. The methanolic extract solution is transferred quantitatively through the glass funnel into the graduated flask and the flat-bottomed flask rinsed with

methanol. The solution in the graduated flask is mixed by gentle shaking. The temperature of the flask is adjusted to 20°C and the contents made up to the mark with methanol. The graduated flask is stoppered and allowed to stand for 1 hour at 0°C to precipitate the hop waxes. A filter funnel, fitted with a dry fluted filter paper, is placed in the neck of a 50 ml graduated flask. The cold methanolic extract solution is poured without stopping into the fluted filter paper. During the filtration the filter funnel is covered with a clock glass. The filtrate in the graduated flask (not made up to volume) is attemperated to 20°C. This is the stock solution.

Determination of Total Resin 10.0 ml of the stock solution is pipetted into a clean, dry (kept for at least 30 minutes in a desiccator) and tared 100 ml flat-bottomed flask (neck not greased). The methanol is distilled off using a rotary evaporator at about 70°C under slight vacuum. When the residue in the flask is dry it is removed from the rotary evaporator and attached to a vacuum adaptor. The residue in the flask is dried at 70°C for 6 minutes under a high vacuum (less than 15 Torr). The vacuum is released and using the water pump air is briefly drawn through the flask. The flask is dried carefully with a soft cloth, then kept for 30 minutes in a desiccator and weighed.

Calculation

$$\text{Total Resin \% w/w} = \frac{\text{Weight of residue (g)} \times 625}{\text{Initial weight of extract taken (g)}}$$

The results are given to one place of decimals.

Accuracy = $\pm 2\%$ (Coefficient of Variation).

Determination of Soft Resin 10.0 ml of methanolic stock solution, 5 ml of methanol (preferably by dispenser) and 40.0 ml of n-hexane are mixed together in a glass centrifuge tube. 10.0 ml of 0.1N hydrochloric acid solution is added, (preferably by dispenser). The centrifuge tube is stoppered and

shaken vigorously for about 30 seconds by hand or for 5 minutes using a shaker, after which it is centrifuged for 2 minutes at 2000 rpm to separate the phases. 30 ml of the clear upper hexane phase is pipetted into a clean, dry, (kept for at least 30 minutes in a desiccator) and tared 100 ml flat-bottomed flask (neck not greased). Using a rotary evaporator, the hexane is distilled off under slight vacuum at 70°C. The flask is removed from the rotary evaporator and attached to a vacuum adaptor. The residue in the flask is dried at 70°C for 6 minutes under a high vacuum (less than 15 Torr). The vacuum is slowly released and using the water pump air is drawn briefly through the flask.

The flask is dried carefully with a soft cloth, then kept for 30 minutes in the desiccator and weighed.

Calculation

$$\text{Soft Resin \% w/w} = \frac{\text{Weight of residue (g)} \times 833}{\text{Initial weight of extract taken (g)}}$$

The results are given to one place of decimals.

Accuracy = ± 3% (Coefficient of Variation)

Determination of Conductometric Value 10 ml of stock solution is pipetted into a wide-necked Erlenmeyer flask or tall beaker followed by 40 ml of dimethylsulphoxide-ethanol mixture. A stirring bar is added and the flask or beaker stirred using a magnetic stirrer. The measuring cell of the automatic titrator is immersed to the correct depth in the liquid. It may be necessary to add more dimethylsulphoxide-ethanol mixture. The burette containing lead acetate solution is placed so that the fine tip is immersed 3 to 4 cm into the test solution. The automatic titrator is switched on and the plot recorded. The intersection of the straight lines for salt precipitation and addition of excess lead acetate solution is the equivalence point. In order to obtain a significant number of points on the straight

line for the addition of lead acetate solution, the titration is continued beyond the equivalence point.

Manual Titration The lead acetate solution is added in 0.25 ml portions and the conductivity recorded after each addition. The titration is continued past the point of inflection until a straight line has been obtained. A graph of conductivity against ml of lead acetate solution is plotted. From the graph the volume of lead acetate solution used for precipitation is employed to calculate the Conductometric Value.

$$\text{Conductometric Value \% w/w} = \frac{\text{Calculation } 5.895 \times T \times \% \text{ lead acetate}}{\text{Initial weight (g)}}$$

where T = Titre of lead acetate solution.

The results are given to one place of decimals.

Accuracy = $\pm 4\%$ (Coefficient of Variation).

Determination of β -Fraction

Calculation

$$\beta\text{-Fraction \% w/w} = \text{Soft Resin} - \text{Conductometric Value}$$

The results are given to one place of decimals.

Determination of Hard Resin

Calculation

$$\text{Hard Resin \% w/w} = \text{Total Resin} - \text{Soft Resin}$$

The results are given to one place of decimals.

Normal Values	% w/w				
	Standard	Special	Super	Extra	Pure Bitter Substances
Total resin*	33-37	43-47	52-58	71-79	> 80
Soft resin	26-32	34-42	42-52	57-71	68-86
Conductometric Value	10-15	13-23	16-29	21-39	26-46
β -Fraction	23-22	17-28	20-35	28-47	34-54
Hard resin	4-9	5-11	6-13	8-17	10-19

* According to Zurich agreement

It was attempted to limit the standardisation by Conductometric Value to types with 15, 30 and 40% Conductometric Values.

Evaluation

Reporting of the Conductometric Value and β -Fraction as percentages of the total resin content allows certain conclusions to be drawn on the variety and growing region of the raw or extracted hops. Furthermore, the ratio of the percentage of hard resin to total resin allows an evaluation of the degree of ageing. Generally in hop extracts a hard resin content of over 17% can be considered as high. It must be remembered when interpreting the results of the hard resin determination that the coefficient of variation is high (approx. $\pm 10\%$).

Note

During the whole of the analytical process, the effects of light, oxygen and heat should be minimised.

References

Pfenninger, H., Schur, F. and Senn, R. Schweiz. Brauerei-Rdsch. 1973, 84, 183-190.

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6.3.4

DETERMINATION OF SPECIFIC HOP COMPOUNDS AND THEIR DERIVATIVES

(International Method)

Principle

Individual components such as α -, iso- α -, humulinic-, β - and δ -acids are separated on an ion-exchange resin column on the basis of their different pK-values, by elution with an acetic acid-methanol gradient. The eluted fractions containing the individual components are collected and their concentrations measured by spectrophotometric measurement of the extract solution at the absorption maximum.

Reagents

Sodium hydroxide solution, 1 N

Hydrochloric acid solution, 0.1 N, 2 N

n-Hexane, analytical reagent

Methanol, analytical reagent

Ethyl ether, peroxide-free

Acetic acid, glacial, ultra analytical reagent

Aqueous methanol solution, 80% v/v

Sodium Sulphate, anhydrous, analytical reagent

QAE-Sephadex, A 25 (40-120 μ)

Aqueous silver nitrate, 2% w/v

Apparatus

Magnetic stirrer

Piston pump (e.g. Milton Roy)

Uvicorder for 280 nm with flow-through cuvette and recorder

UV-Spectrophotometer

Shaker

Vacuum rotary evaporator

Angle centrifuge about 2500 rpm

Glass columns: a) 40 cm long, internal diameter 2 cm

b) 35 cm long, internal diameter 0.5 cm

Erlenmeyer flasks with ground glass joint 500 ml, 150 ml

Standard flasks, 20 ml, 25 ml, 50 ml, and 100 ml

Pipettes, 1 ml, 20 ml and 50 ml

Centrifuge tubes with screw caps, 100 ml (e.g. Hawk Glassblowing, D-85 Nurnberg, Pickheimerstrasse 51)

Measuring cylinder, 100 ml.

Connecting tubes. The various parts of the apparatus are connected using Teflon tubing of internal diameter 1 mm. (for glass-Teflon changeover "Swagelok^R" connectors are recommended).
(See fig I).

Method

Pretreatment of ion-exchange resin

20 g of QAE Sephadex A25 is suspended in 1N sodium hydroxide solution and allowed to stand overnight. The swollen gel is poured into the glass column (40 x 2 cm) and washed with 1N sodium hydroxide until a test for chloride with silver nitrate solution is negative. The column is washed with distilled water until neutral and then with acetic acid until the eluate shows no absorption at 275 nm. The Sephadex resin is re-suspended in a solution of 80% acetic acid in 80% methanol and transferred to an Erlenmeyer flask.

Preparation of an elution gradient

A 500 ml Erlenmeyer flask is filled with acetic acid. 75 ml of 80% methanol contained in a 150 ml Erlenmeyer flask is placed on a magnetic stirrer. Both flasks are connected with ground glass connections and Teflon tubing. (See Fig 1.)

Pretreatment of column.

A column (35 x 0.5 cm) is filled with the prepared resin suspension until the height of the resin bed is 30 cm. The column is then washed with 80% aqueous methanol until neutral.

Preparation of sample solution

Hops, Hop powder Hop Pellets and Hop Extract Powder

10 g of finely ground hops, hop pellets, or hop powder product are transferred to a tared centrifuge tube using a wide mouth funnel and weighed accurately. 20 ml of methanol and 100 ml of ether are added by pipette. The centrifuge tube is closed with a screw cap and placed in the mechanical shaker. After shaking the tube for 30 minutes, it is opened cautiously and 40 ml of 0.1N hydrochloric acid added. The centrifuge tube is capped and shaken for a further 10 minutes at maximum speed, removed and allowed to stand for 10 minutes. An aliquot of the ether phase is diluted with methanol to give a solution containing a maximum of 0.5 mg of α -acids per ml, 0.5 mg β -acids per ml and 1 mg of iso- α -acid per ml.

Hop extracts and iso-extracts

The extract is heated to 65° for 30 minutes and mixed well with a glass rod. 1 g is weighed accurately and dissolved in 100 ml of methanol. Any material not dissolved is removed by

centrifugation. The solution is diluted if necessary so that it contains a maximum of 0.5 mg of α -acids per ml, 0.5 mg of β -acids per ml and 1 mg of iso- α -acid per ml.

Wort and beer

To 20 ml of wort or beer in a centrifuge tube is added 20 ml of 2N hydrochloric acid solution and 30 ml of n-hexane. The centrifuge tube is closed with a screw cap and shaken for 30 minutes on a mechanical shaker. After centrifuging for 10 minutes at 2000 rpm, 20 ml of the hexane phase is removed by pipette. (If an emulsion is formed it can be broken by removing the aqueous phase and adding anhydrous sodium sulphate). The hexane solution is evaporated to dryness at a maximum of 40°C using a rotary evaporator. The residue is dissolved in 1 ml of methanol and transferred quantitatively to the column.

Chromatographic separation and quantitative estimation of the individual components.

1 ml of sample is pipetted onto the column and the glass wall of the column rinsed with a little methanol. The components are eluted from the column using the solvent gradient at a flow rate of 50 ml/hour. The pen-recorder is started and the fractions of hop components collected in volumetric flasks (β -acids in 20 ml, α -acids in 25 ml, iso- α -acids and δ -acids each in 100 ml volumetric flasks). The solutions in the flasks are made up to the mark with 80% methanol and after mixing the absorbance is measured against 80% methanol in a 1 cm cell using a spectrophotometer.

	<u>Wavelength (n m)</u>	<u>E_{1 cm}^{1%}</u>	<u>Retention time (minutes)</u>
β-acids	340	232	15
α-acids	290	225	30
Humulinic acid	270	362	60-70
Iso-α-acids	275	277	95-100
δ -acids	280	276	190-200

When the hop components have been eluted the column is regenerated by washing with acetic acid for 30 minutes followed by 80% methanol until the eluate is neutral.

Calculation

The hop components are calculated using the formula

$$\frac{A \times V_1 \times V_2}{E_{1 \text{ cm}}^{1\%} \times g} \quad \text{where}$$

A = Absorbance found

V₁ = Volume of eluate in ml

V₂ = Sample volume in ml

E_{1 cm}^{1%} = Absorbance of a 1% solution of hop components in a cell of 1 cm length

g = weight of sample in g

Example : Estimation of α-acids

Solution of sample = 0.1125 g hop extract in 200 ml methanol

Eluate : = 25 ml

Absorbance at 290 nm = 0.183

E_{1 cm}^{1%} = 225

$$\alpha\text{-acids} = \frac{0.183 \times 25 \times 200}{225 \times 0.1125} = 36.1 \text{ \% w/w}$$

The results for hops and hop products are given as % w/w to one

decimal place.

For worts and beers the results are given in mg/l to one decimal place.

The accuracy is dependent on sample, on the type of components being estimated and on their concentration in the sample.

Notes

The acetic acid should be examined from time to time for optical purity.

The individual fractions containing eluted hop components should be measured as quickly as possible in the spectrophotometer as there is a danger of decomposition of these components.

References

Otter, G.E. Silvester, D.J. and Taylor, L : J. Inst. Brewing, 1972, 78, 57-64.

Ganzlin, G : Brauwissenschaft, 1975, 28, 231-239.

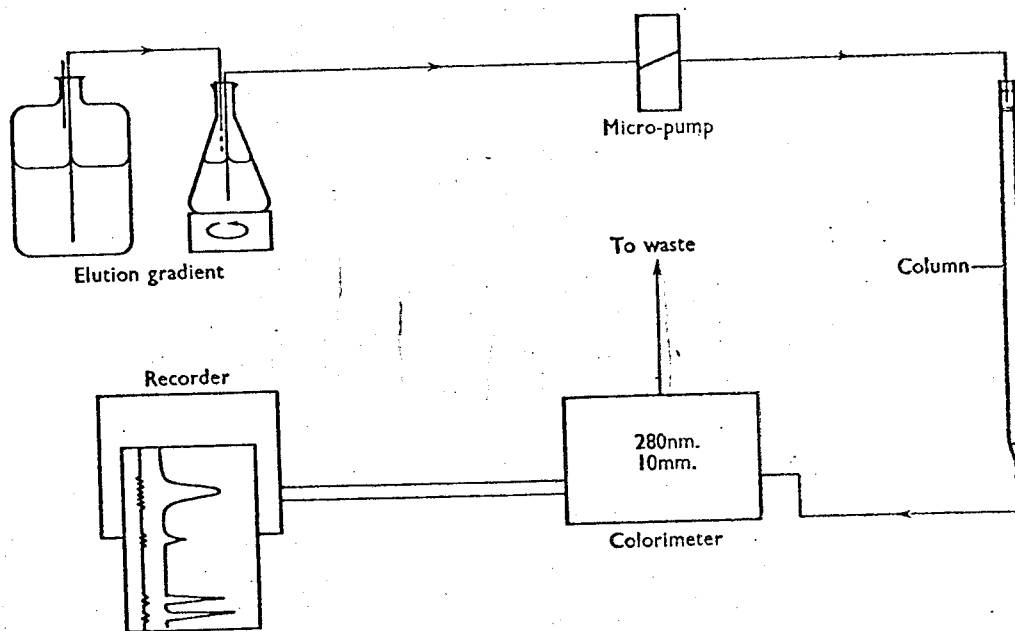


Fig. 1.—Flow diagram of the chromatography and analyser systems.

6.3.5. DETERMINATION OF ALPHA ACIDS IN HOPS, HOP POWDERS,
HOP PELLETS AND HOP EXTRACTS
(International Method)

Principle

Alpha acids are quantitatively separated from other hop constituents on QAE Sephadex A-25 ion exchange resin and measured by UV spectrophotometry at 280 nm in 10% acetic acid-aqueous methanol. The method requires small volumes of reagents and analytical time is about one hour.

Reagents

QAE Sephadex A-25 resin, chloride form (Note 4)

Acetic acid, glacial, ultra analytical reagent.

Aqueous methanol (80%). (800ml analytical reagent grade methanol made up to 1000 ml with distilled water).

Acetic acid solutions, prepared fresh daily:

5% acetic acid in 80% methanol (v/v)

10% acetic acid in 80% methanol (v/v)

Benzene, analytical reagent.

Toluene, analytical reagent.

Sodium hydroxide solution, 1N

Aqueous silver nitrate, 2% w/v

Apparatus

Chromatography column, 0.5 cm. ID x 30 cm (Kontes K-420500, size CD 3005, with column fittings, teflon tubing and flaring tool). (Note 3)

Teflon tubing 0.053 in. ID (Kontes K-42074).

Valve, 3-way (Kontes K-423700).

Adapter, septum (Kontes K-423560).

Septa (Kontes K-749102).

Coupling, straight (Kontes K-423550).

Apparatus (Continued)

Bottles, 4 Oz. with screw cap, several for use as solvent reservoirs.

2 syringes, 1 ml, with # 24 gauge needles. The sample syringe must be accurately calibrated, e.g. Hamilton 500 or 100 μ l UV spectrophotometer, preferably scanning.

Pressure source, preferably nitrogen cylinder with 2-step regulator.

Septum for 4 oz bottle (cut from neoprene sheet or stopper, with 2 small holes for teflon tubing and nitrogen inlet). (See Fig.1)

Conversion of resin and preparation of column

The QAE Sephadex A-25 is converted from the chloride form to the acetate form using the following procedure. 5g of the dry powder is suspended in 50 ml of 1N sodium hydroxide and allowed to stand overnight. The swollen resin is transferred into an all glass column which is large enough to hold all the resin. The resin is washed with 1N sodium hydroxide until the effluent is free of chlorides when tested with silver nitrate-nitric acid. The resin is washed with distilled water until neutral and with glacial acetic acid until the eluted acid has an absorbance of zero at 275 nm when read against a glacial acetic acid blank. As much acetic acid as possible is drained off and the resin slurried with 80% acetic acid in 80% methanol (v/v). A small glass wool plug is inserted in the bottom of a column (30 x 0.5 cm) to support the resin. The resin is added as a thin slurry and the column is built up a little at a time using very low pressure to remove excess liquid after each addition of slurry. This resin shrinks considerably in strong acetic acid. Since the column is packed with the resin in a shrunken state, 2 to 3 cm space at

the top of the column must be allowed. A small glass wool plug is placed at the top of the column which is then inverted and connected as shown in Fig. I. Acetic acid is removed by washing with 80% methanol in the opposite direction to which the column was packed. The flow of methanol is very slow and it may be necessary to invert the column once or twice to expand the resin into the column void and to establish the correct flow rate of 50 ml/hour. The column is washed with 80% methanol until free of acetic acid. This will take about 15 minutes at 50 ml/hour. The column is now ready for use.

Sample preparation

Hop Extract

The hop extract sample is heated to 65°C for 30 minutes and mixed well with a glass rod. 1g of the extract is weighed accurately onto a piece of tared glassine paper 1" x 1" and transferred to a 100 ml volumetric flask, taking care not to get any sample on the neck of the flask. 50 ml of 80:20 methanol water and 5 glass beads are added, the flask stoppered and shaken for 30 minutes on a wrist action shaker. The solution is made up to volume with 80:20 methanol water, mixed and the solids allowed to settle.

Hops, Hop Powders & Hop Pellets

5.000 ± 0.001g of freshly ground hops, hop powder or hop pellets is transferred quantitatively to a 250 ml extraction bottle and 100 ml benzene or toluene added. The container is tightly stoppered and shaken vigorously on a mechanical shaker. The suspension is allowed to stand until the solids are sufficiently settled to permit the withdrawal of a clear aliquot (this should not exceed 1 hr) or if necessary centrifuged at

200 rpm for 5 minutes. This solution contains the extract from 50 mg hops, hop pellets or hop powders per ml.

Method

Three labelled reservoir bottles are filled with 80% methanol, 5% glacial acetic acid and 10% glacial acetic acid, taking care that the eluting solvents do not come into contact with the rubber. For 80% methanol the flow rate is adjusted to between 50 and 60 ml/hour (Note 2). The 3-way valve is turned to open the channel from the injection septum to the column and the methanol water extract (containing 0.5 to 1.0 mg of α -acids) slowly injected. Usually 0.25 ml of the extract is adequate. The needle is withdrawn after a period of at least 5 seconds. 0.5 ml of 80% methanol is slowly injected with a clean syringe to wash the sample onto the column. The 3-way valve is turned to open the channel between the reservoir and the column. Approximate volumes for each elution step are given in Table 1.

Table 1

<u>Acetic Acid %</u>	<u>Minimum Volume</u>	<u>Approximate Volume needed</u>	<u>Terminating abs. 280 nm</u>	<u>Compound eluted</u>
0	10 ml	10 ml	---	Interferences & Solvent
5	20	25	0.05	Interferences & beta-acids
10	35	50	0.05	Alpha-Acids

The column is eluted with 10 ml of 80% aqueous methanol and the eluate discarded. The eluting solvent is changed to 5% acetic acid by turning off the 3-way valve, turning off the nitrogen supply, and then exchanging reservoir bottles. 20 ml of the 5% acetic acid eluate is collected in a graduated cylinder. A 5 ml

fraction is then collected, mixed and the absorbance measured in a spectrophotometer at 280 nm and 340 nm against a 5% acetic acid blank. (The acid spectra of α - and β -acids are given in Fig 2.)

If A_{280} is greater than 0.05 and less than A_{340} , then the β -acids are not completely eluted. Elution with 5% acetic acid is continued and each 5 ml fraction checked at 280 nm and 340 nm until A_{280} is less than 0.05 or A_{340} begins to increase. If A_{280} is greater than 0.05 and greater than A_{340} , then the α -acids are beginning to elute. Elution with 5% acetic acid is stopped and the eluting solvent changed to 10% acetic acid. The last fraction of the 5% acetic acid is mixed with the 10% acetic acid eluate.

If A_{280} is less than 0.05 then 35 ml of 10% acetic acid eluate are collected in a graduated cylinder fitted with a glass stopper. 5 ml fractions are collected and the A_{280} of each fraction measured against a 10% acetic acid blank until the A_{280} is less than 0.05. The total combined volume of 10% acetic acid eluate and any 5% eluate which contained α -acids is measured and recorded. The combined eluate is well mixed and the absorbance at 280 nm determined against a blank of 10% acetic acid solution. The column is regenerated at the end of each analysis.

Regeneration of the column.

The column is washed with 100 ml glacial acetic acid. At this stage the resin will have a dry, channelled appearance. This will disappear as the resin expands when the column is filled with methanol. The column is inverted without disturbing the column connections and 80% methanol passed up through the column until it emerges from the column exit. The flow rate is

very slow. The column connections are exchanged and 80% ethanol passed down through the column in the opposite direction to the initial flow of acetic acid using very low pressure. The resin should expand and the flow rate rapidly improve. The column is washed with 80% methanol until it is free of acetic acid. The column is returned to its original position and the connections are again exchanged. The column is now ready for use.

Calculation

Weight of sample injected w is calculated from

$$w = \frac{W \times V_1}{V_2}$$

where W = weight of sample (g)

V_1 = volume of extract injected (ml)

V_2 = volume of extract (ml)

Example

Sample weight = 1.0g, volume of extract = 99.5 ml, volume injected = 0.25 ml

$$w \text{ sample on column} = \frac{1}{99.5} \times 0.25$$
$$= 0.0025g.$$

The % α -acids in the sample is calculated from

$$\% \alpha\text{-acids} = \frac{A_{280} \times V_3}{E \times w}$$

where A_{280} = Absorbance at 280 nm

V_3 = Total volume of eluate (ml)

w = Weight of sample on column (g)

E = Extinction coefficient. $E_{1\text{cm}}^{1\%}$ in acid at 280 nm

Example

Sample weight = 0.0025g, Volume of eluate = 50 ml

Absorbance at 280 nm = 0.189

$$\% \alpha\text{-acids} = \frac{0.189 \times 50}{221 \times 0.0025} = 17.1$$

Notes

1. The eluate may degas when coming off the column; this will not affect the results.
2. The flow rate is important. The volumes needed to elute alpha-acids were determined using a flow rate of 60 ml per hour. If the flow rate is very different, the volumes needed will be different.
3. The chromatography column and fittings may be obtained from: Kontes Glass Company, Vineland, NJ 08360; or Kontes of California, 3054 Teagarden St., San Leandro, CA 94577.
4. The American supplier of QAE Sephadex A-25 is : Pharmacia Fine Chemicals, 800 Centennial Ave., Piscataway, NJ 98854.
5. Glass beads are very uniform in size and should displace about 0.1 ml per bead. The total volume would then be 99.5 ml instead of 100 ml.

References.

American Society of Brewing Chemists. Report of the Sub-committee on Hop Analysis. Proc. Amer. Soc. Brew Chemists, 1970, 244.

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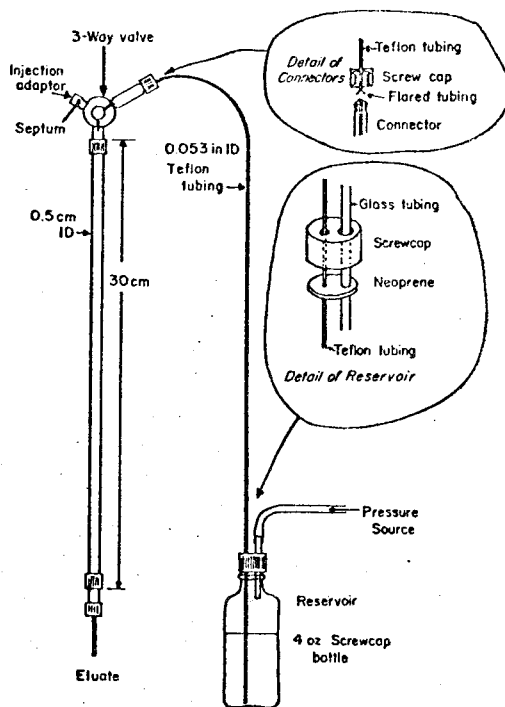


Figure 1. Sephadex Chromatographic Column and Reservoir.

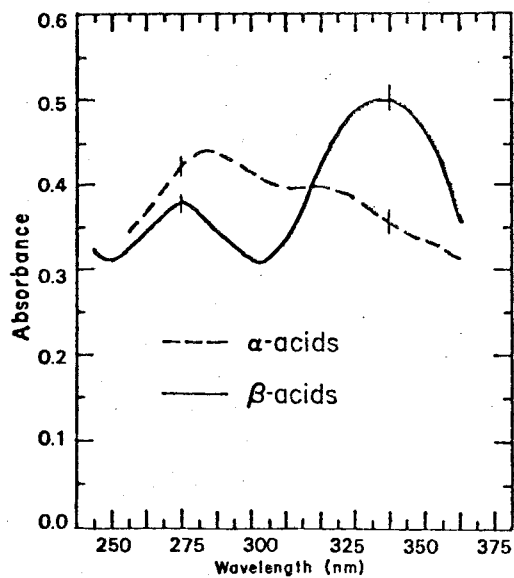


Figure 2. UV Spectra of α -acids in 10% acetic acid and of β -acids in 5% acetic acid.

6.3.6 Estimation of Iso-alpha-acids in Isomerized Hop Extracts

(International Method)

Principle

Iso- α -acids are quantitatively separated from the hop constituents on QAE Sephadex A-25 ion exchange resin and measured by UV spectrophotometry at 280 nm in 60% acetic acid-aqueous methanol. The method requires small volumes of reagents and analytical time is about $1\frac{1}{2}$ hours.

Reagents

QAE Sephadex A-25 resin, chloride form. (note 4)

Acetic acid, glacial, ultra analytical reagent.

Aqueous methanol (80%) (800 ml analytical reagent grade methanol made to 1000 ml with distilled water)

Acetic acid solutions, prepared fresh daily:

40% acetic acid in 80% methanol

60% acetic acid in 80% methanol

Apparatus

See 6.3.5. Hops, Hop Powders, Hop Pellets and Hop Extracts.

Conversion of resin and preparation of column.

See 6.3.5. Hops, Hop Powders, Hop Pellets and Hop Extracts.

Sample Preparation

The isomerized extract is heated to 65°C for 30 minutes and mixed well with a glass rod. 1 g of the extract is weighted accurately onto a piece of tared glassine paper 1" x 1" and transferred to a 250 ml flask taking care not to get any sample on the neck of the flask. 100 ml of methanol (80%) and 5 glass beads are added, the flask stoppered and shaken for 30 minutes on a wrist action shaker.

Method

Three labelled reservoir bottles are filled with 80% methanol, 40% glacial acetic acid and 60% glacial acetic acid, taking care that the eluting solvents do not come into contact with the rubber.

For 80% methanol the flow rate is adjusted to between 50 and 60 ml/hour (note 2). The 3-way valve is turned to open the channel from the injection septum to the column. A sample of the methanol water extract containing 0.5 to 1.0 mg of iso- α -acid (usually 0.25 ml) is slowly injected and the needle withdrawn after a period of at least 5 seconds. 0.5 ml of 80% methanol is then slowly injected with a clean syringe to wash the sample onto the column and the 3-way valve turned to open the channel between the reservoir and the column.

Approximate volumes for each elution step are given in Table 1.

TABLE I

<u>% Acetic Acid</u>	<u>Min. vol.</u>	<u>Approx. Vol. Needed</u>	<u>Terminating Abs. 280 nm</u>	<u>Compound Eluted</u>
0	10 ml	10 ml	—	Interferences & solvent
40	40 ml	50 ml	0.05	β -acids, α -acids humulinic acid Interferences
60	50 ml	60 ml	0.05	Iso- α -acids

The column is eluted with 10 ml of 80% aqueous methanol and the eluate discarded. The eluting solvent is changed to 40% acetic acid by turning off the 3-way valve, turning off the nitrogen supply and exchanging reservoir bottles. 40 ml of the 40% acetic acid eluate is collected in a graduated cylinder. A 5 ml fraction is then collected, mixed and the absorbance measured at 280 nm against a 40% acetic acid blank.

If A_{280} is greater than 0.05, then the elution with 40% acetic is continued and each 5 ml fraction checked at 280 nm until, A_{280} is less than 0.05, or A_{280} begins to increase.

If A_{280} is less than 0.05 then 50 ml of 60% acetic acid eluate are collected in a volumetric flask. 5 ml fractions are collected and the A_{280} of each fraction measured against a 60% acetic acid blank until the A_{280} is less than 0.05. The total combined volume of 60% acetic acid eluate and any 40%

eluate which contained iso- α -acids is measured and recorded. The combined eluate is well mixed and the absorbance at 280 nm is determined against a blank of 60% acetic acid solution. The column is regenerated at the end of each analysis.

Regeneration of the column.

See 6.3.5. Hops, Hop Powders, Hop Pellets and Hop Extracts.

Calculation

Weight of sample injected w is calculated from

$$w = \frac{W \times V_1}{V_2}$$

where W = Weight of sample (g)

V_1 = Volume of extract injected (ml)

V_2 = Volume of extract (ml)

Example

Sample weight = 1.0178 g, Volume of extract = 100 ml

Volume injected = 0.25 ml

$$\text{Sample on column (w)} = \frac{1.0178}{100} \times 0.25 = 0.00255\text{g}$$

The % iso- α -acids in the sample is calculated from

$$\% \text{ iso-}\alpha\text{-acids} = \frac{A_{280} \times V_3}{E \times w}$$

where A_{280} = Absorbance (280 nm)

V_3 = Total volume of eluate (ml)

w = Weight of sample on column

E = Extinction coefficient $E_{1\%}^{1\text{cm}}$ in acid at 280 nm is 250

Example

Sample weight = 0.00255g, Volume of eluate = 50 ml

Absorbance at 280 nm = 0.245

$$\% \text{ iso-}\alpha\text{-acids} = \frac{0.245 \times 50}{250 \times 0.0025} = 19.3$$

Notes

See 6.3.5. Hops, Hop Powders, Hop Pellets and Hop Extracts.

Substitute iso- α -acids for α -acids.

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2. Otter, G.E., Silvester, D.J., and Taylor, L. J. Inst. Brew. 78: 57. (1972).



EUROPEAN BREWERY CONVENTION (E.B.C.)

Secretariat General, Registered Office: Rotterdam

MINUTES OF THE 50th MEETING OF THE EBC ANALYSIS COMMITTEE, BRUGES,
11th DECEMBER 1979

In attendance

M. Moll (F) : Chairman
G.H. Ulenberg (NL) : Secretary-General of EBC
Mrs. Marjolein van Wijngaarden (NL) : Assistant Secretary of EBC

Members in alphabetical order of countries represented (s.s.t.t.)

A	J. Püspök	GB	J.R. Hudson
B	R. van der Beken		P.A. Martin
	D. Eyben	N	S. Lie
CH	H.B. Pfenninger	NL	B.W. Drost
D	H. Weyh		W.J. Klopper
DK	P. Gjertsen	P	M.J. Cardoso
	I. Rosendaal	S	R. Westelius
E	M. Ferrer	SF	S. Home
	S. Martin		M. Sopo

Also present (s.s.t.t)

L. Narziss (D) : President of EBC
J. van Strien (NL) : accompanying B.W. Drost
M. Benard (F) : representing R. Scriban

Apologies for not attending were received from (s.s.t.t.)

R. Tressl (D) - J. Maier (D) - R. Scriban (F) - T. Zangrando (I) -
L. van der Stappen (NL)

1. Introduction

The Chairman opened the meeting at 14.00 h and welcomed all members, especially the past Chairman of the Committee, Prof. Enari, the President of EBC, Prof. Narziss and the Secretary-General of EBC, Mr. Ulenberg.

On behalf of all members of the Committee, the Chairman offered a farewell present to Prof. Enari as a token of gratitude for all he had accomplished for the Committee, both during his chairmanship and as a member. In a short speech Prof. Enari thanked everybody for their co-operation, and he praised the very active participation. Thereupon Prof. Enari left the meeting.

It was announced that the past President of EBC, Mr. Clutterbuck, was unable to attend the meeting.

2. Minutes of the 49th Meeting

The Minutes of the 49th Meeting, held in Berlin (West) on 26th May 1979, were approved.



EUROPEAN BREWERY CONVENTION (E.B.C.)

Secretariat General, Registered Office: Rotterdam

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3. Reports of Subcommittees

./.

See Appendix I for detailed reports on the meetings of the various Subcommittees, held on 10th and 11th December 1979.

The following observations were made at the discussions:

- Dr. Pfenninger announced that he would like to be discharged as Chairman of the Subcommittee Analytica Microbiologica at the next meeting of the Analysis Committee.
The publication of part II of Analytica Microbiologica has been delayed through lack of assistance and the absence of generally accepted methods.
- Mr. Sopo joined the Diacetyl Subcommittee. ASBC did not agree with the gaschromatographic method. Capture electron detection is now tried.
- The Subcommittee on Flavour did not meet this time because there had not been enough time to study all methods distributed by ASBC. Mr. Ulenberg explained the present situation concerning the Flavour Terminology Working Group. In its meeting of October 1979, the Council of EBC decided to discontinue this Group; results had already been published. However, the Council considered holding a meeting on flavour, especially on beer flavour, most important and therefore had decided to organise a Symposium in the autumn of 1981 (replacing the planned Workshop Session), resulting in the publication of another Monograph.
Mr. Ulenberg furthermore announced that in 1980 a Symposium on flavour would be organised in Holland, and in 1981 one in Athens (ACS), but that these would be dedicated to flavour in a broader sense.
Anyone wishing to have more information on flavour was requested to contact Mr. Moll.
- Mr. Rosendal succeeded Dr. Hudson as Chairman of the Subcommittee on Hops and Hop Extracts. The Chairman thanked Dr. Hudson for all he had done for the Analysis Committee during the last fourteen years of his chairmanship.
Dr. Hudson would draw up a press report announcing the availability of the new standard extract, to be distributed among the brewing press by the Secretariat of EBC.
- During discussions of the Subcommittee on Malt Analysis, Prof. Narziss offered to discuss any problems with the Bühler-Miag Mill during a meeting with the manufacturers in January 1980. Members were requested to send their complaints to Prof. Narziss before the middle of January 1980.
- A new Subcommittee on Packaging Control was formed (see also item 8a), with as members Messrs. Rosendal (Chairman) and Drost (assisted by Mr. Van Strien); Mr. Kremkow (VLB) would be asked to participate.
- A new Subcommittee on Husk Determination in Barley was formed (see also item 8d), with as members Messrs. Püspök (Chairman), Van der Beken and Klopper. Prof. Schildbach should be asked to join this Subcommittee.

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4. Analytica III

Dr. Pfenninger announced that the following methods were being printed and that distribution was foreseen towards the middle of January 1980:

atomic absorption - sodium in beer - potassium in beer - magnesium in beer - calcium in beer - copper in beer - iron in beer - zinc in wort - filter aids - moisture contents of filter aids - calcination loss - density of wet filterbed - pH of water suspension - soluble iron - effect on odour and taste - DLFU Bühler-Miag Mill - determination of pregerminated grains in barley.

The chapters on Hops and Sampling of Raw Materials would be published in a next Amendment.

It was announced that over 1000 copies of Analytica III had been sold.

5. Alcohol Tables

The Chairman was of the opinion that it would be most suitable for EBC to adopt an alcohol table in the line of an international institution, like ISO or IUPAC, and moreover in agreement with ASBC.

Mr. P.A. Martin suggested to adopt the tables issued by the EEC; in Belgium and The Netherlands these tables had already been adopted. At the request of Mr. Rosendal, Mr. Moll agreed to distribute all reactions on alcohol tables received so far, among the members of the Main Committee, as well as the EEC tables, to be sent to him by Mr. Eyben.

A decision could be taken at the next meeting in May 1980.

6. Future work

Future work, agreed to be executed by members of the various Subcommittees, was summarised:

- | | |
|-----------------------------|--|
| a. Analytica Microbiologica | : Methods of part II are to be finalized by Messrs. Leedham, Martin and Germain. |
| b. Diacetyl | : New interlaboratory tests on gaschromatography. |
| c. Enzymic Activities | : Special work on beta-glucan, waiting for endopeptidase tablets. |
| d. Extract of Adjuncts | : Improvement of methods, draft for various rice varieties. |
| e. Filter Aids | : Description of methods to be published. |
| f. Flavour | : Next meeting reporting on methods. |
| g. Germination | : Next meeting reporting on information received from Mr. Deymié. |
| h. Hops and Hop Extracts | : No future work foreseen. |

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- i. Instrumental Colour Measurement : Five samples to be sent to the members for collaborative testing.
- j. Malt Analysis : Two series of two malts to be distributed for interlaboratory test of Tepral method.
- k. Oxalate : New samples to be sent.
- l. Sampling of Raw Materials : No future work.
- m. Sugars and Carbohydrates : Reporting on caloric value measurements.
- n. CO₂ determination : Two series of two beers to be distributed for collaborative tests.

7. Date and place of next meeting

Prof. Enari had invited the members of the Analysis Committee to hold their next meeting in Helsinki, from 5-7 May 1980, on the occasion of the official opening of the new Biotechnical Laboratory. The invitation was gratefully accepted.

8. Other business

- a. On the suggestion of Mr. Rosendal, a Subcommittee on Packaging Control was established. Members: Messrs. Rosendal (Chairman) and Drost (assisted by Mr. Van Strien); Mr. Kremkow (VLB) would be asked to participate.
Mr. Rosendal made reference to a report on this subject, already distributed by him, and stressed the need for standard methods. It was agreed that first of all a comparison of already published material would be made, and that at the next meeting Mr. Rosendal would hand in a 3 or 4 year-plan with a list of priorities.
Mr. Eyben made reference to a Working Group in CBMC dealing with this subject.
- b. In spite of the proposal of the Brewing Research Foundation to adopt the ISO-method for determination of the moisture content in barley (130° instead of 105°C), it was decided to stick to the EBC rules, described in Analytica. The Chairman would inform the BRF.
- c. On his question on the accuracy of haze meters, Mr. Ulenberg was referred to the lecture given by Mr. Leedham at the Amsterdam 1977 Congress, and to an article published in Brauerei Rundschau (Jan./Feb. issue) by Mr. Sigrist. In any case, the apparatus should be calibrated regularly and the black colour repainted frequently.
- d. At the request of Mr. Püspök, a new Subcommittee on Husk Determination in Barley was formed. Members: Messrs. Püspök (Chairman), Van der Beken and Klopper. Prof. Schildbach would be asked to participate.

Mr. Moll thanked all members for their attention and co-operation, and closed the meeting at 16.45 h.

Zoeterwoude, January 1980

APPENDIX II

TYPE OF ANALYSIS: TERMIAMI REFINED MAIZE GRITS
 NUMBER OF LABORATORIES: 17

NO. OF LABORATORY, X-VALUE, Y-VALUE, ETC.
 #1 104.3 104.5
 #3 104.3 104.7
 #4 104.2 104.3
 #5 102.7 99.4
 #6 103.6 103.5
 #7 103.7 103.8
 #8 102.8 103.0
 #9 101.9 102.4
 #10 103.4 103.6
 #11 103.5 103.5
 #12 103.8 104.1
 #13 103.9 104.3
 #14 103.1 103.5
 #15 102.7 103.1
 #16 102.5 102.7
 #17 103.3 102.9
 #18 103.3 103.5
 #20 103.4 103.5

Minutes of the 50th Meeting
 of the EBC Analysis Committee,
 Bruges, 11th December 1979

see Appendix I, (d)

X-VALUES: NO DIXON-OUTLIERS
 LABORATORY: 5, Y-VALUE DIXON-OUTLIER
 X-MIN, X-MAX, Y-MIN, Y-MAX
 #23, 107.23, 107

COEFFICIENT OF VARIATION X-VALUES 0.60%
 COEFFICIENT OF VARIATION Y-VALUES 0.63%
 ST. = 0.876 MEAN X = 103.36 MEAN Y = 103.53
 F-RATIO: 24.07 CONFIDENCE CIRCLE RADIUS: 0.434

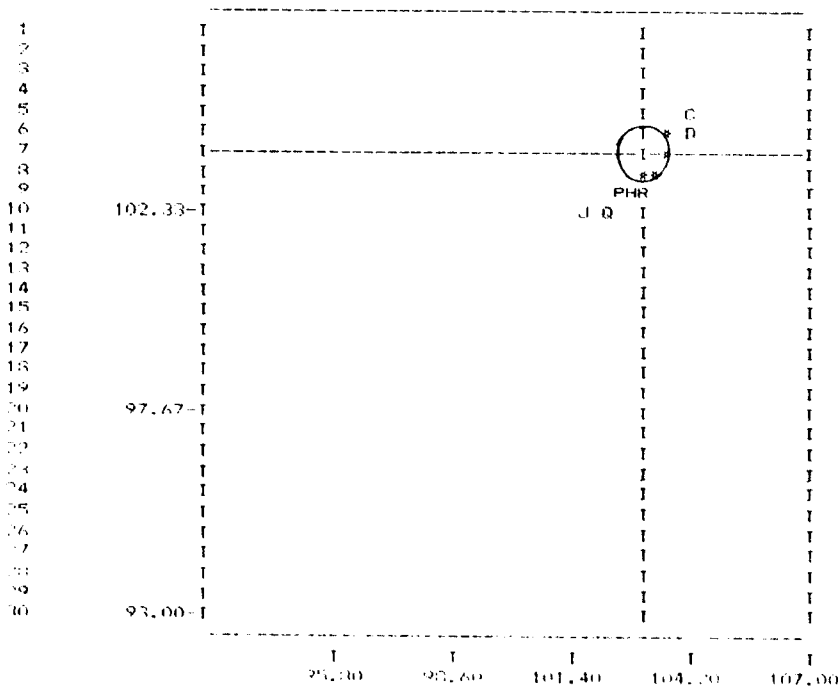
NUMBER OF LABORATORIES: 17

TEST FOR: TERMIAMI REFINED MAIZE GRITS	AVERAGE AMOUNT (X+Y)/2	PRECISION SR	SYSTEMATIC SB	NO. OF LABORATORIES
	103.47	0.18	0.61	17

COEFFICIENT OF VARIATION IN TOTAL DATA 0.63%

LABORATORY NO.	SYMBOL
1	A
3	C
4	D
6	F
7	G
8	H
9	J
10	K
11	L
12	M
13	N
14	O
15	P
16	Q
17	R
18	S
20	U

* INDICATES TWO OR MORE RESULTS



POINTS IN CONFIDENCE CIRCLE $= 0.44$
 THIS CIRCLE $=$



EUROPEAN BREWERY CONVENTION (E.B.C.)

Secretariat General, Registered Office: Rotterdam

MEMBERS OF SUBCOMMITTEES OF THE ANALYSIS COMMITTEE, WHO ARE NOT
A MEMBER OF THE MAIN COMMITTEE

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U.S.A.

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ENSIA
Chaire de Malterie-Brasserie
Eaux et Boissons Gazeuses
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France

G. GANZLIN
Lupofresh Allfeld & Egloff
Senefelderstrasse 8-14
Abhofach
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Bodenkultur & Pflanzenbau
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Prof. M. VERZELE
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Laboratorium voor Organische
Scheikunde
Krijgslaan 271
Gent
Blok S.4.
Belgium

BRYGGERIINDUSTRIENS FORSKNINGSLABORATORIUM

FORSKNINGSVEIEN 1 - OSLO 3 - TELEFON 69 58 80

EBC Analysis Committee

OSLO, 29.11.79

RESULTS OF COLLABORATIVE TEST OF ALPHA AMYLASE ACTIVITY-PHARMACIA TABLETS

Enclosed please find the results of our latest test. I have treated the data in four ways. Firstly, all data of malt N and O are treated in the usual way. Secondly, malt N and O are corrected for deviations in the 9th std. malt. Thirdly, only the "trained" laboratories, members of the Enzymic Subcommittee are included. Fourthly, the latter group corrected by means of the std. malt.

Survey of results:

1) No. of laboratories:	20		
Coeff. of variation:	<u>N</u>	<u>O</u>	
	25.56%	30.91%	
2) No. of laboratories:	12		
Coeff. of variation:	<u>N</u>	<u>O</u>	
	17.04%	22.18%	
3) No. of laboratories:	7		
Coeff. of variation:	<u>N</u>	<u>O</u>	
	17.64%	27.38%	
4) No. of laboratories:	5		
Coeff. of variation:	<u>N</u>	<u>O</u>	
	16.50%	23.12%	

With kind regards,

Sincerely yours

pp Sturla Lie

Encs.

α -Amylase Collaborate Test

October–November 1979

Activity in IU/1.000.000

BRYGGERIINDUSTRIENS
FORSKNINGSLABORATORIUM

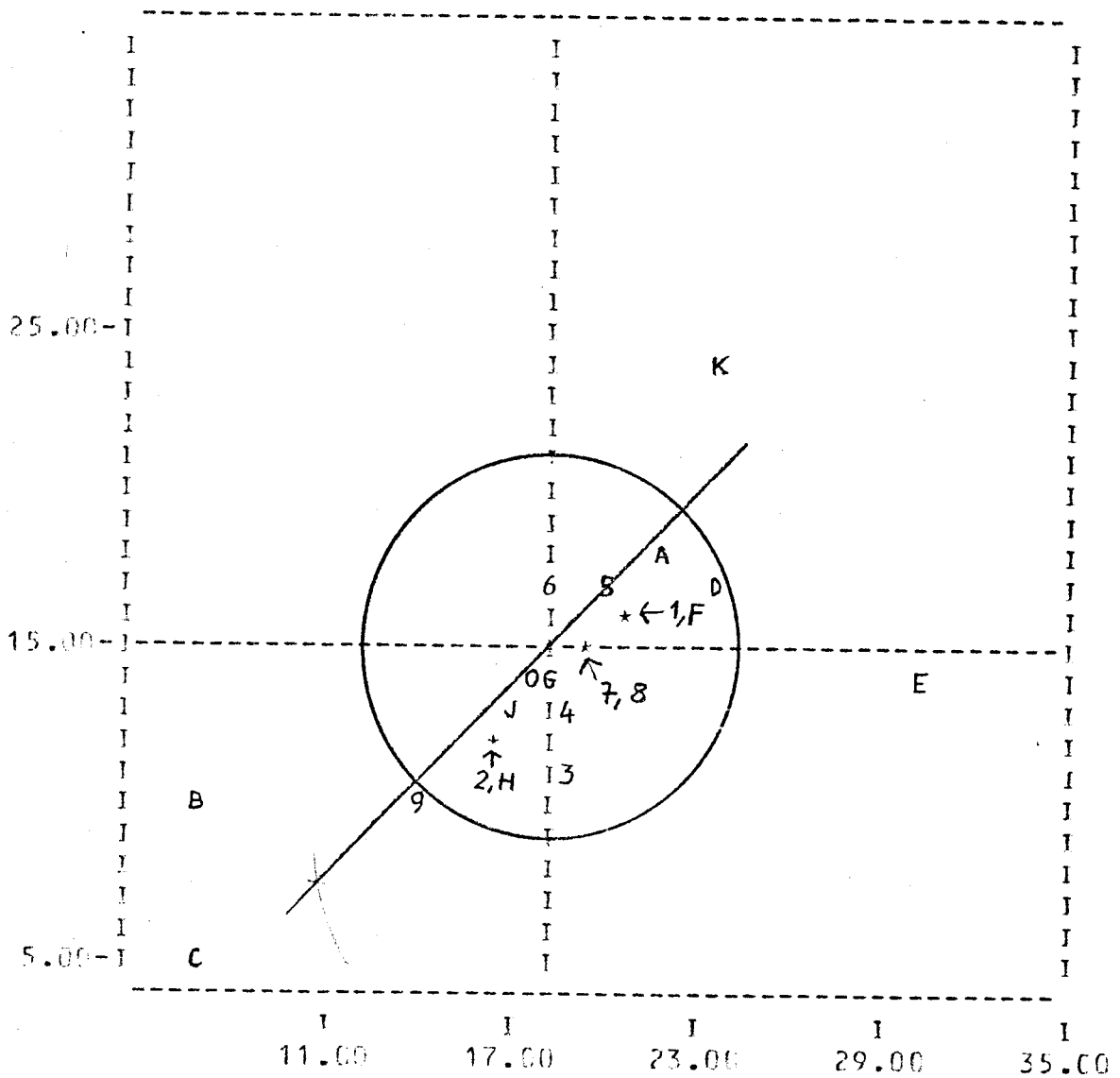
No.	Navn	NX	NY	OX	OY	9th std. malt	^E 620 x 1000 Blank	Comments
1	van der Beken	20.8	16.3	37.5	50.0	29.0	12	
2	Cardoso	16.5	13.0	31.5	42.5	21.5	23	
3	Drawert	19.0	12.0	22.5	35.0	26.0	25	
4	Drost	19.0	14.0	33.0	48.0	22.5	27	
5	Eyben	20.0	17.5	28.0	42.0	-	-	
6	Ferrer	18.7	17.9	33.0	33.4	-	39	
7	Gjertsen	19.6	15.2	33.7	48.7	26.3	5	
8	Home	19.5	15.5	29.5	46.0	22.5	13	
9	Hudson	14.3	11.0	30.0	28.0	20.5	12	
O = 10	Klopper	18.0	14.2	30.4	41.0	22.3	36	
A = 11	Lie	22.0	18.5	41.0	62.5	-	6	
12	Martin, P.A.	7.3	10.3	15.0	22.5	-	20	
C = 13	Martin, S.	6.8	5.3	13.0	14.5	-	40	
D = 14	Moll	23.8	17.9	41.2	63.1	27.8	29	
E = 15	Pfenninger	30.3	14.8	49.7	39.4	25.0	20	1)
F = 16	Püspök	21.0	17.0	36.0	43.0	24.0	12	
G = 17	Rosendal	18.5	15.0	33.1	48.6	26.3	26	
H = 18	Scriban	16.8	12.2	25.4	39.3	24.3	21	
19	Sopo							
J = 20	van der Stappen	17.3	13.5	29.0	35.8	-	-	
K = 21	Tressl	24.0	24.5	35.1	44.0	-	-	
22	Westelius	(18.3)	(14.3)	(42.0)	(48.0)	(26.3)	(7)	
23	Weyh							
24	Zangrando							

1) Deviating method
not included in
corrected data.

α -amylase Collaborative test
Results corrected by means of
9th std. malt

No.	Navn	NX	NY	OX	OY
1	van der Beken	17.5	13.7	31.6	42.1
2	Cardoso	18.7	14.8	35.8	48.3
3	Drawert	17.9	11.3	21.1	32.9
4	Drost	20.6	15.2	35.8	52.1
5	Eyben				
6	Ferrer				
7	Gjertsen	18.2	14.1	31.3	45.2
8	Home	21.2	16.8	32.0	49.9
9	Hudson	17.0	13.1	35.7	33.4
10	Klopper	19.7	15.6	33.3	44.9
11	Lie				
12	Martin, P.A.				
13	Martin, S.				
14	Moll	20.9	15.7	35.2	55.4
15	Pfenninger				
16	Püspök	21.4	17.3	36.6	43.8
17	Rosendal	17.2	13.9	30.7	45.1
18	Scriban	16.9	12.3	25.5	39.5
19	Sopo				
20	van der Stappen				
21	Tressl				
22	Westelius	(17.0)	(13.3)	(39.0)	(44.6)
23	Weyh				
24	Zangrando				

ALFA AMYLASE NX-NY TOTAL DATA



COEFFICIENT OF VARIATION X-VALUES 27.88%

COEFFICIENT OF VARIATION Y-VALUES 26.32%

ST = 6.000

MEAN X = 18.66 MEAN Y = 14.78

F-RATIO: 5.81

CONFIDENCE SIRCLE RADIUS: 6.0961

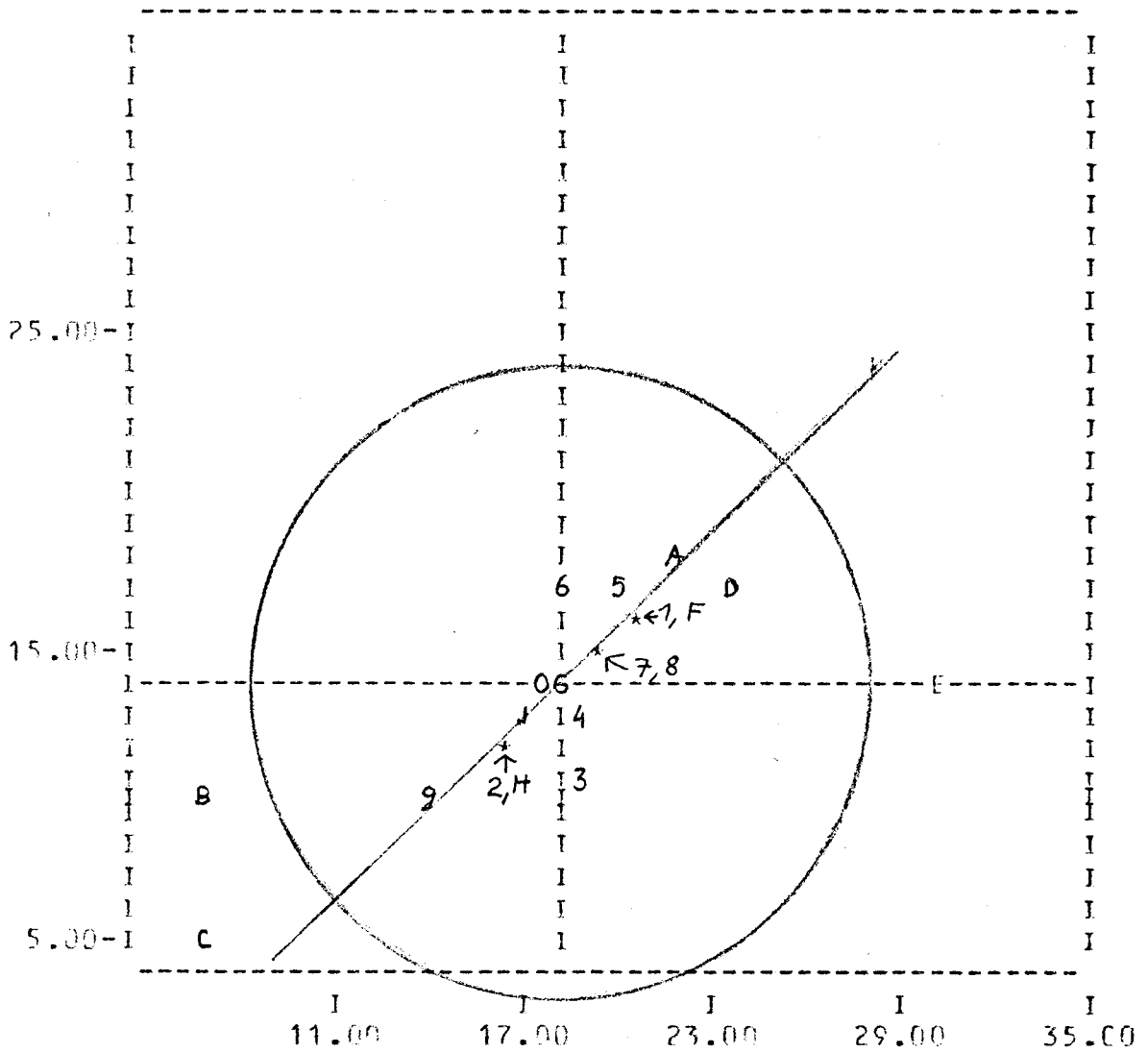
AVERAGE AMOUNT (X+Y)/2	PRECISION SB	SYSTEMATIC SB
---------------------------	-----------------	------------------

16.72	2.49	3.86
-------	------	------

COEFFICIENT OF VARIATION IN TOTAL DATA 29.56%

ALFA-AMYLASE NX-NY

TOTAL DATA
- LAB. 20



COEFFICIENT OF VARIATION X-VALUES

27.88%

COEFFICIENT OF VARIATION Y-VALUES

34.38%

ST = 5.661

MEAN X = 18.66

MEAN Y = 13.50

F-RATIO: 1.93

CONFIDENCE SIRCLE RADIUS: 9.9677

AVERAGE AMOUNT

PRECISION

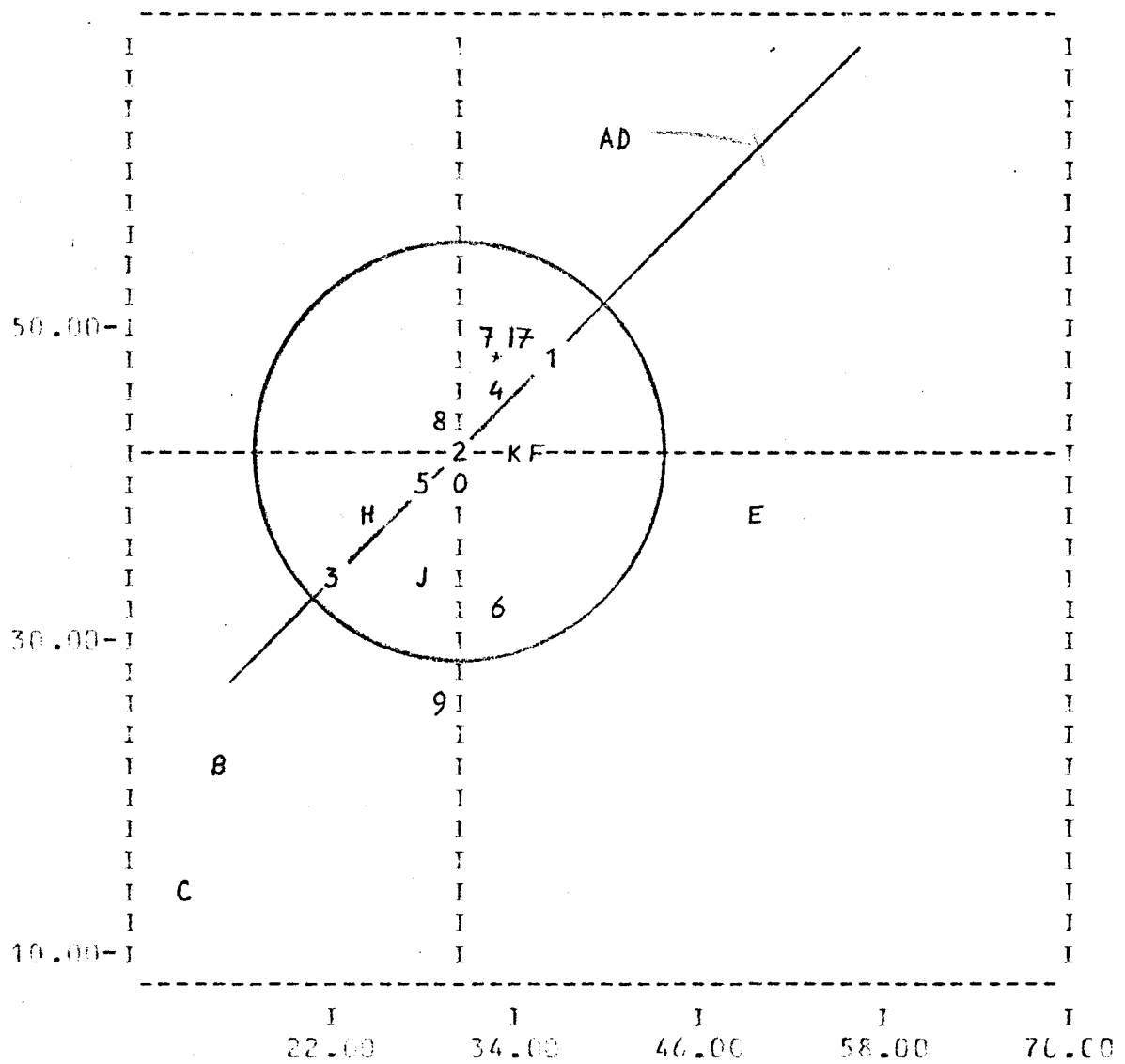
SYSTEMATIC

$(x+y)/2$

SB

SB

COEFFICIENT OF VARIATION IN TOTAL DATA



COEFFICIENT OF VARIATION X-VALUES 26.91%

COEFFICIENT OF VARIATION Y-VALUES 28.15%

ST = 13.252

MEAN X = 31.38 MEAN Y = 41.36

F-RATIO: 5.61

CONFIDENCE SIRCLE RADIUS: 13.6912

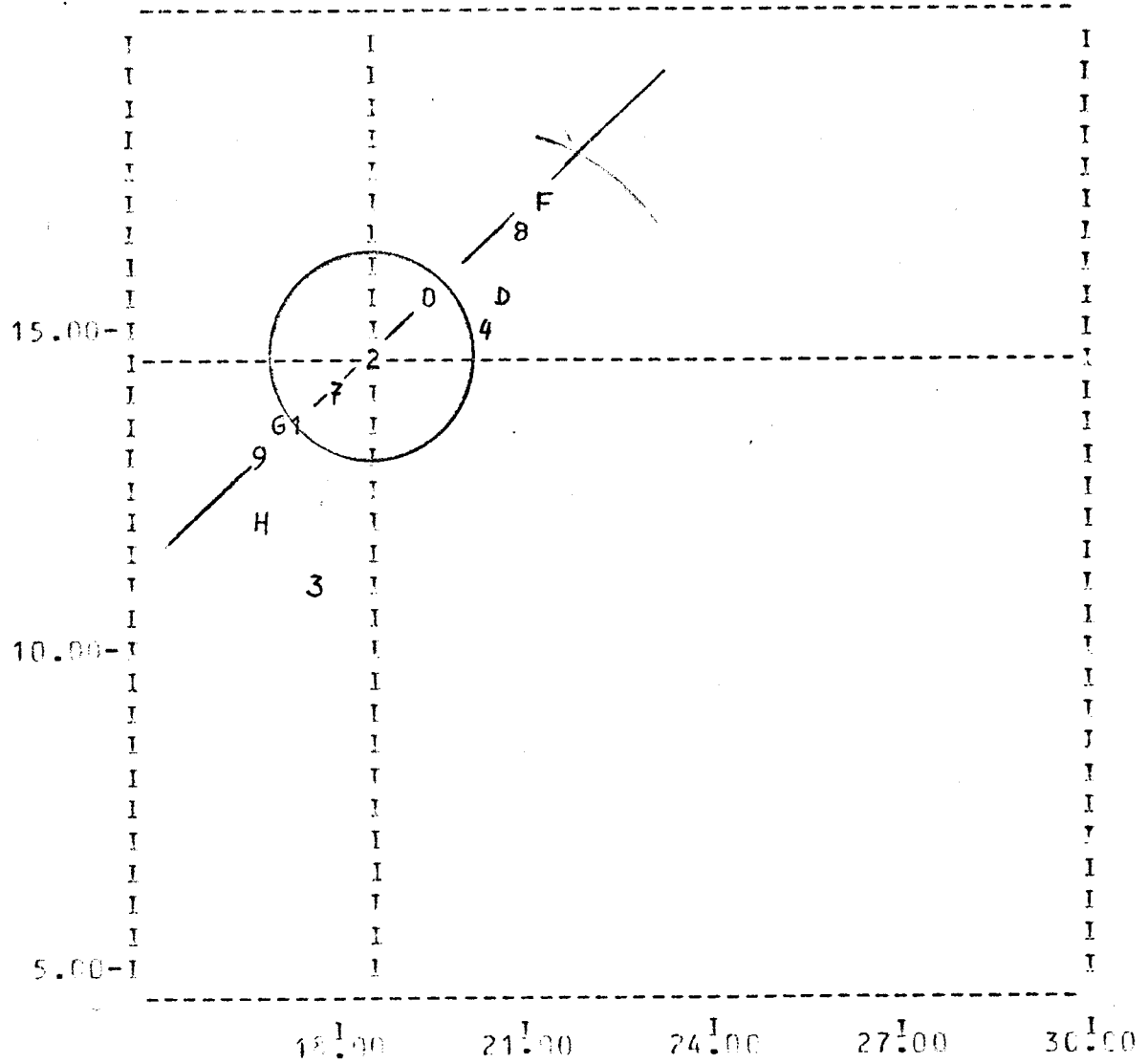
AVERAGE AMOUNT (X+Y)/2	PRECISION SB	SYSTEMATIC SB
---------------------------	-----------------	------------------

36.37	5.59	8.50
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COEFFICIENT OF VARIATION IN TOTAL DATA 30.91%

2)

ALFA-AMYLASE NX-NY CORRECTED BY MEANS OF 9TH STD. MALT



COEFFICIENT OF VARIATION X-VALUES 9.15%

COEFFICIENT OF VARIATION Y-VALUES 12.24%

ST = 2.397

MEAN X = 18.93 MEAN Y = 14.48

F-RATIO: 14.31

CONFIDENCE SIRCLE RADIUS: 1.5509

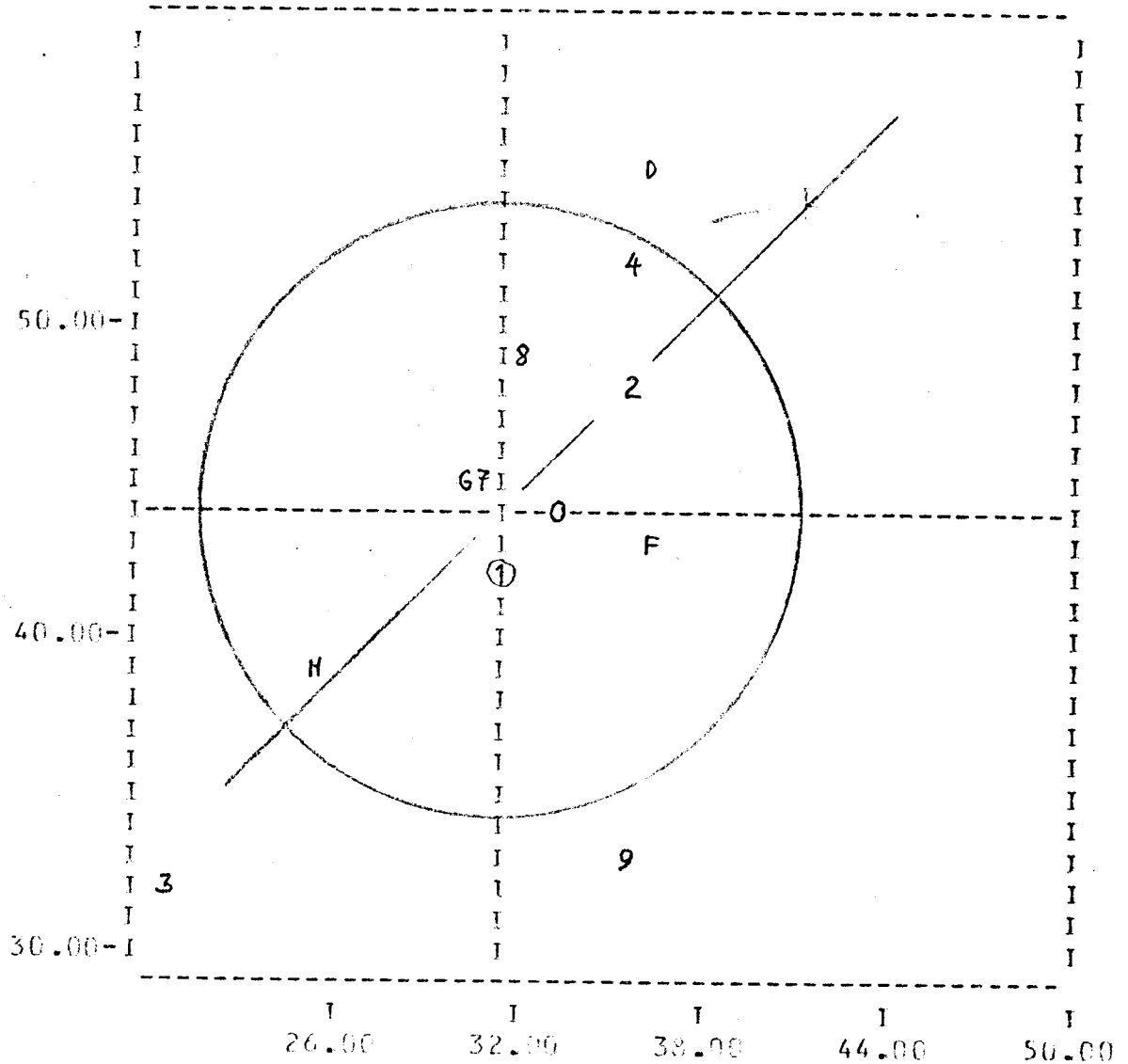
AVERAGE AMOUNT (X+Y)/2	PRECISION SB	SYSTEMATIC SB
---------------------------	-----------------	------------------

16.71	0.63	1.63
-------	------	------

COEFFICIENT OF VARIATION IN TOTAL DATA 17.04%

2)

ALFA-AMYLASE OX-OY CORRECTED BY MEANS OF 9TH STD. MALT



COEFFICIENT OF VARIATION X-VALUES 14.72%

COEFFICIENT OF VARIATION Y-VALUES 15.34%

ST = 7.276

MEAN X = 32.13 MEAN Y = 44.38

F-RATIO: 3.35

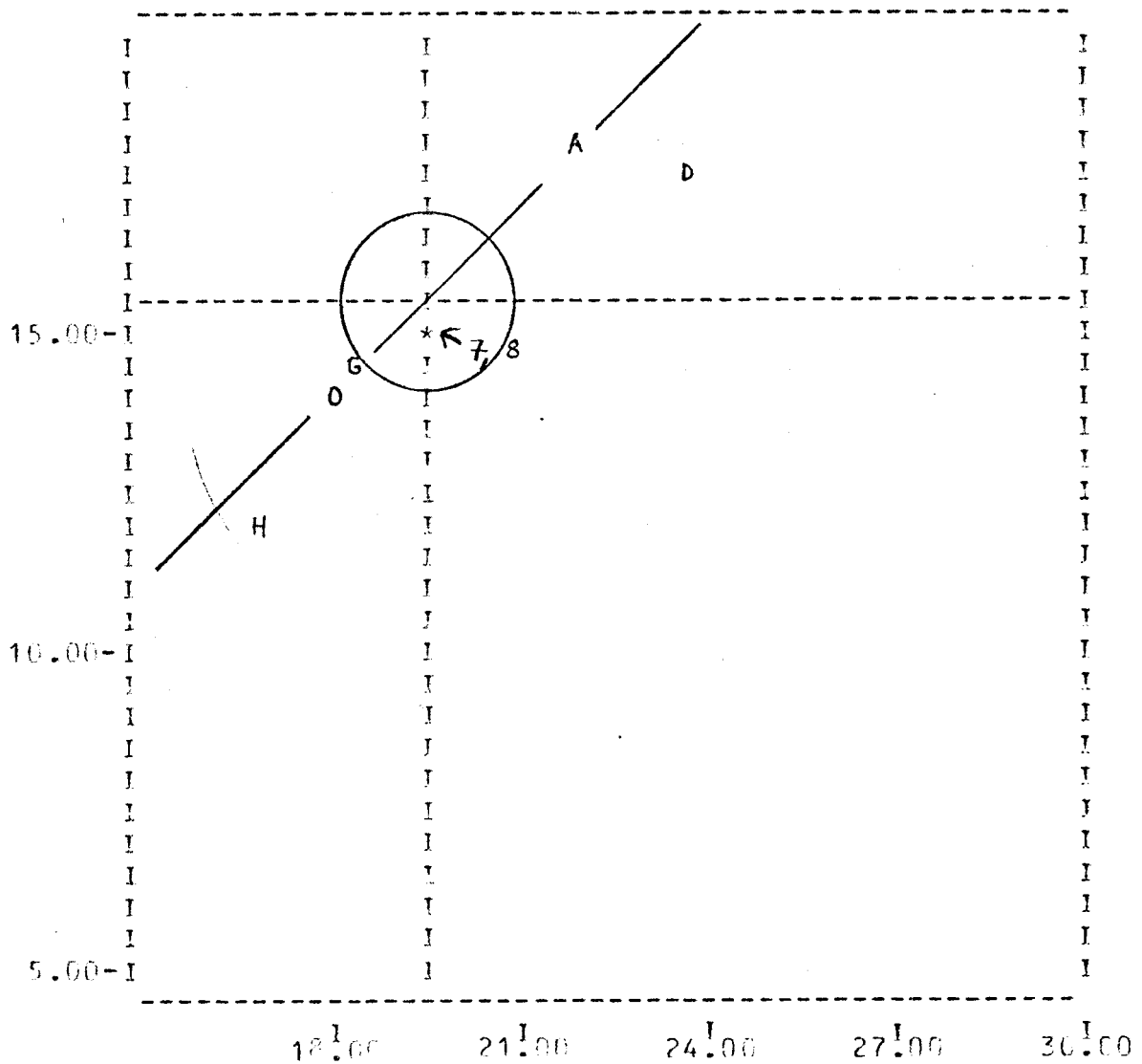
CONFIDENCE SIRCLE RADIUS: 9.7361

AVERAGE AMOUNT (X+Y)/2	PRECISION SB	SYSTEMATIC SB
38.26	3.98	4.31

COEFFICIENT OF VARIATION IN TOTAL DATA 22.18%

3/

ALFA-AMYLASE NX-NY 7 TRAINED LABORATORIES (SUB-COMMITTEE)



COEFFICIENT OF VARIATION X-VALUES 12.21%
 COEFFICIENT OF VARIATION Y-VALUES 13.86%

ST = 3.174

MEAN X = 19.74 MEAN Y = 15.50

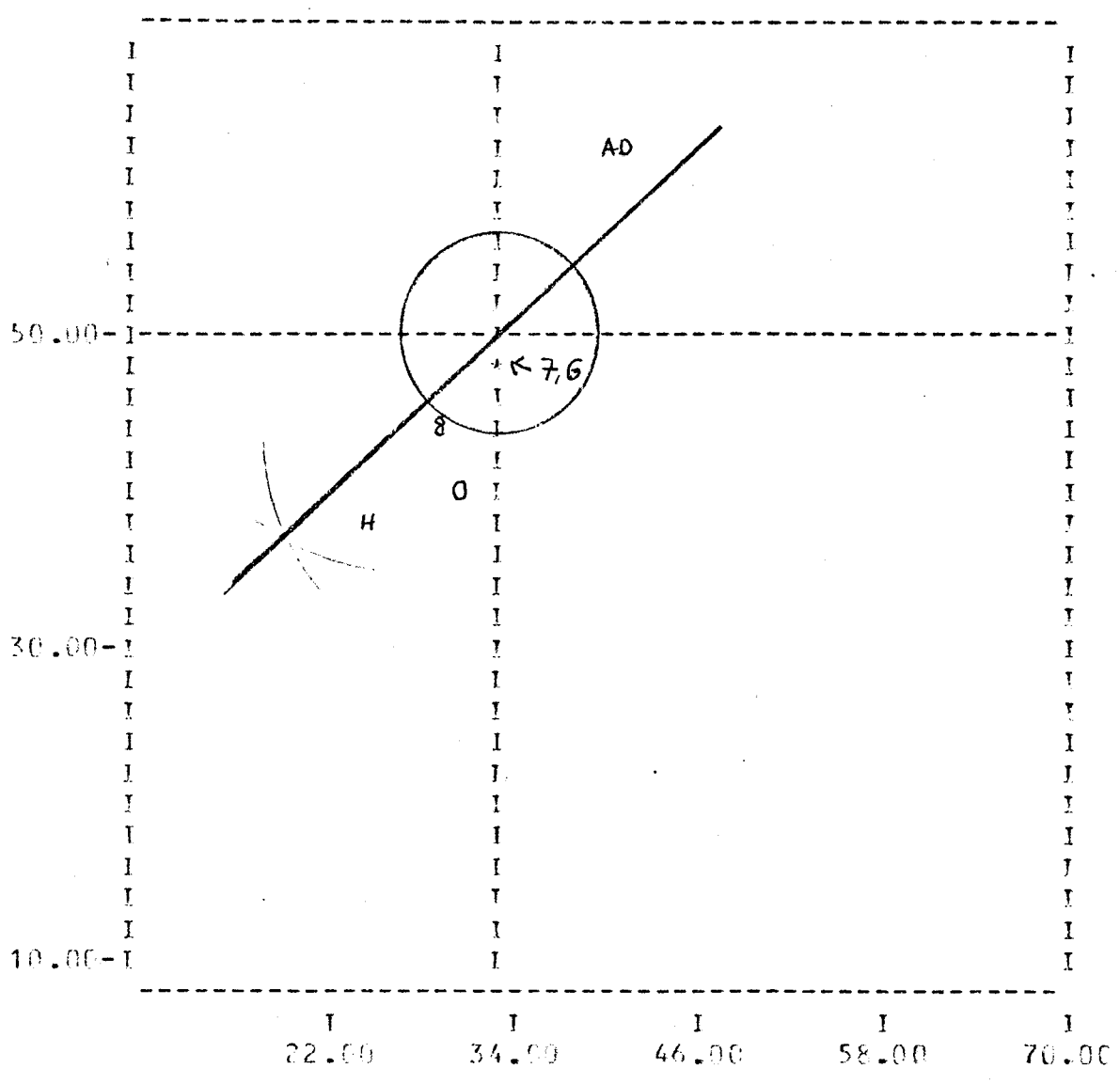
F-RATIO: 28.39

CONFIDENCE SIRCLE RADIUS: 1.4581

AVERAGE AMOUNT (X+Y)/2	PRECISION SB	SYSTEMATIC SB
---------------------------	-----------------	------------------

17.62	0.60	2.20
-------	------	------

COEFFICIENT OF VARIATION IN TOTAL DATA 17.64%



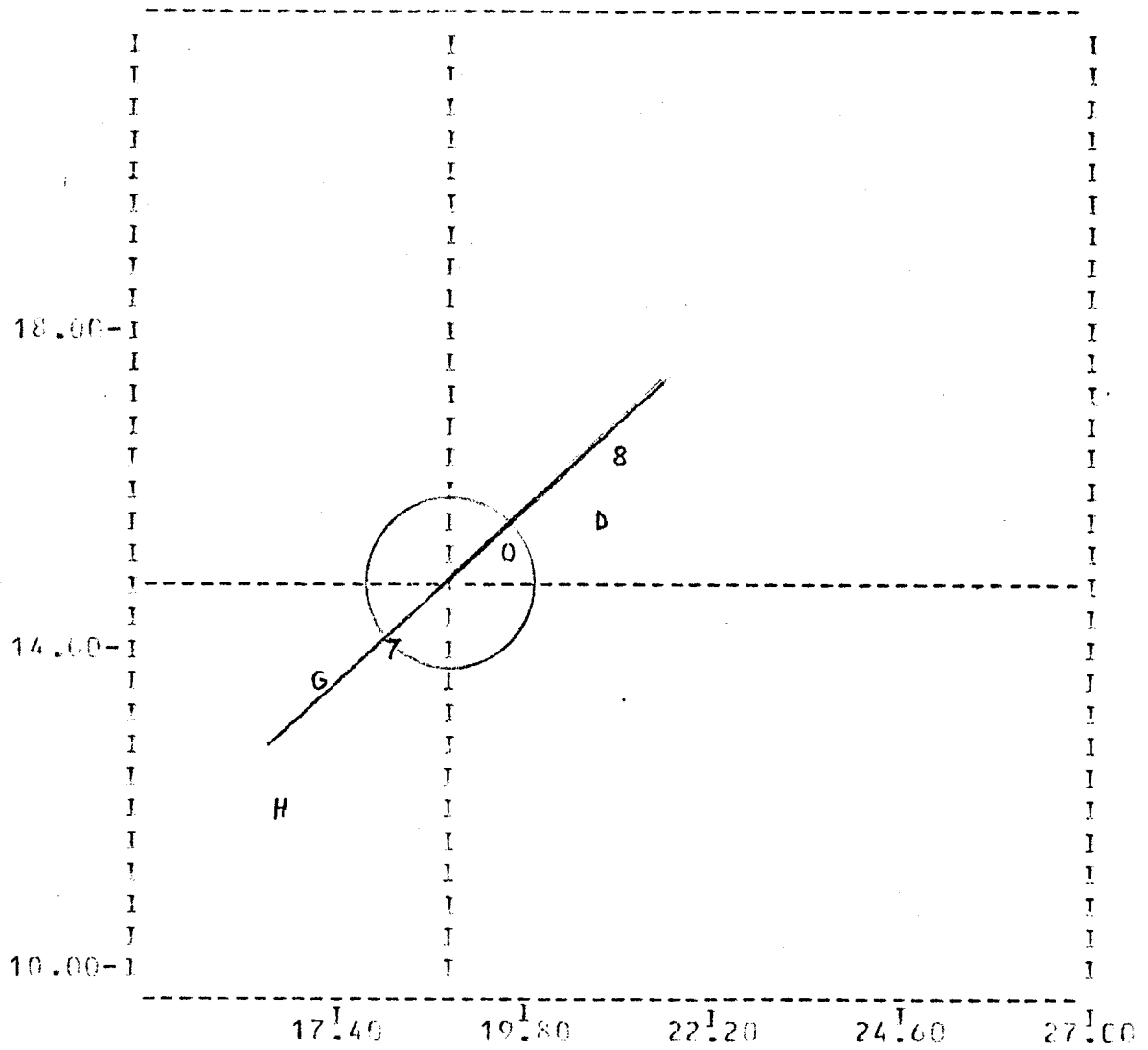
COEFFICIENT OF VARIATION X-VALUES 17.55%
 COEFFICIENT OF VARIATION Y-VALUES 19.07%
 ST = 10.804
 MEAN X = 33.47 MEAN Y = 49.89
 F-RATIO: 14.16
 CONFIDENCE SIRCLE RADIUS: 7.0288

AVERAGE AMOUNT (X+Y)/2	PRECISION SB	SYSTEMATIC SB
41.68	2.87	7.37

 COEFFICIENT OF VARIATION IN TOTAL DATA 27.38%

41

ALFA-AMYLASE NX-NY (SUB-COMMITTEE)
RESULTS CORRECTED BY MEANS OF 9TH STD. MALT



COEFFICIENT OF VARIATION X-VALUES 9.70%

COEFFICIENT OF VARIATION Y-VALUES 10.93%

ST = 2.417

MEAN X = 19.02 MEAN Y = 14.73

F-RATIO: 29.38

CONFIDENCE SIRCLE RADIUS: 1:0916

AVERAGE AMOUNT (X+Y)/2	PRECISION SB	SYSTEMATIC SB
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16.87	0.45	1.63
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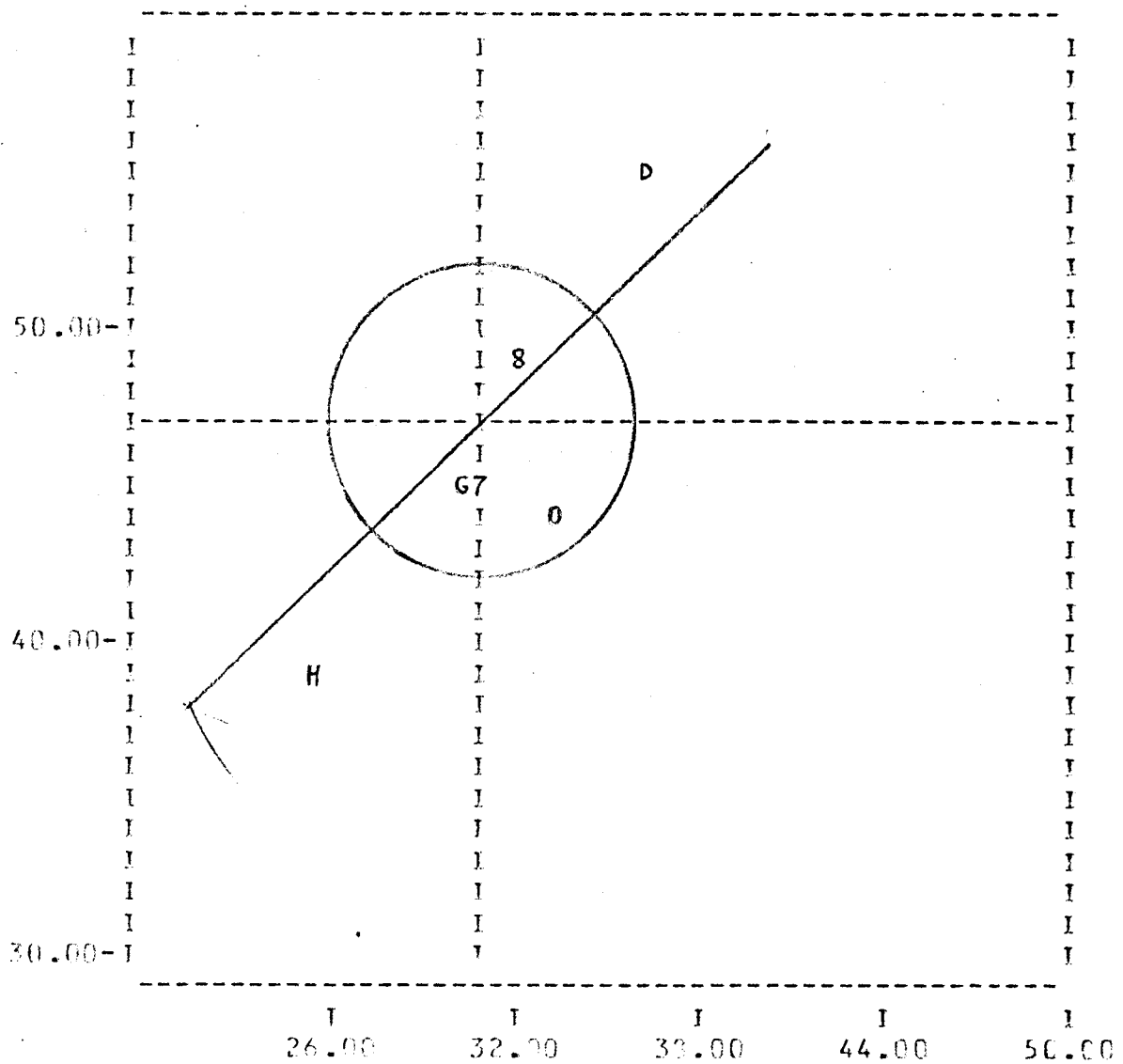
COEFFICIENT OF VARIATION IN TOTAL DATA 16.50%

41

ALFA-AMYLASE OX-OY

SUB COMMITTEE

RESULTS CORRECTED BY MEANS OF 9TH STD. MALT



COEFFICIENT OF VARIATION X-VALUES 11.20%
 COEFFICIENT OF VARIATION Y-VALUES 11.57%

ST = 6.133

MEAN X = 31.50 MEAN Y = 46.67

F-RATIO: 9.40

CONFIDENCE SIRCLE RADIUS: 4.8968

AVERAGE AMOUNT (X+Y)/2	PRECISION SB	SYSTEMATIC SB
---------------------------	-----------------	------------------

39.08	2.00	4.10
-------	------	------

COEFFICIENT OF VARIATION IN TOTAL DATA 23.12%



EUROPEAN BREWERY CONVENTION (E.B.C.)

Secretariat General, Registered Office: Rotterdam

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The Netherlands

Tel. 071 - 814047/814614
Telex: c/o 39390 henb nl

To the Members of the EBC
Analysis Committee

Our ref.: 301/80/U1/mvw
Your ref.:
Please deal with only one subject per letter

ZOETERWOUDE, 31st January 1980

04 FEB. 1980

Minutes of the 50th Meeting
of the EBC Analysis Committee
Bruges, 11th December 1979

Dear Sirs,

We have pleasure in sending you enclosed the Minutes of the 50th Meeting of the EBC Analysis Committee, held at Bruges on 11th December 1979, together with Appendix I and II.

For your information we also enclose two revised sheets belonging to the List of Members, sent to you on 7th January 1980 with covering letter No. 46/80.

The Minutes have been approved by the Chairman of the Committee, Mr. M. Moll.

Yours sincerely,

encls.

G.H. Ulenberg

THE BREWING RESEARCH FOUNDATION

Research Establishment

From J.R. HUDSON, Ph.D., F.R.I.C.
Deputy Director

Lyttel Hall, Nutfield,
Redhill, Surrey.
RH1 4HY

Tel: Nutfield Ridge (073 782) 2272
Telex: 8814000

5th November, 1979

Dear Gail,

Please accept my profuse apologies for not replying to any of your letters for at least 6 months. This has been largely due to the fact that the Director, Dr. Dalgliesh, resigned as from 1 July, so that I have had to spend all my time on Foundation and national Committee matters, with the consequent delay in attending to international matters. In fact, I have tendered my resignation as Chairman of the EBC Sub-Committee on Hops and Extracts, and doubtless a new Chairman will be appointed at the December meeting. I trust that he will prove a better correspondent and more assiduous in his duties than I have been for this past year.

Despite these difficulties, we have finalised the altered methods which are to be included in Analytica, and I enclose copies. I did also submit for publication the note on statistical data which led to our selection of the two modifications of the Otter/Taylor procedure as international methods, and I enclose a copy.

With kind regards,

Sincerely yours,

Dr. Gail Nickerson,
Dept. of Agricultural Chemistry,
Oregon State University,
Corvallis,
Oregon 97331,
U.S.A.

Future Work

A. Mr. Ganzlin will circulate:-

- (i) One sample of a new E.B.C. Standard Extract (pure resin).
- (ii) A & B samples of a standardised extract made from fresh hops.
- (iii) A & B samples of a standardised extract made from stored hops.

These will be analysed by the existing Verzele method in Analytica and the revised version in the paper now circulated.

B. If possible, Dr. Pfenninger will circulate a sample of synthetic isohumulone to be analysed by the "Stepwise" and "Gradient" elution methods.

Next Meeting: 10th December 1979, Bruges, Belgium.

E.B.C. Analysis Committee

Sub-Committee on Hops and Extracts

Minutes of a meeting held in Berlin, 25th May 1979

Present: Mr. G. Ganzlin Dr. H. Pfenninger
Dr. J.R. Hudson (Chairman) Mr. I. Rosendal
Mr. M. Moll
Mr. H. Grant attended on behalf of A.S.B.C.

Dr. J. Maier had apologised for his absence.

I. Minutes of Last Meeting

Mr. Rosendal noted that on 13th June 1978 the Main Committee had transferred responsibility for sampling procedures to a newly formed Sub-Committee which will deal with all brewing materials.

II. Amended Drafts of Methods for Analytica

Attention was drawn to the requirement in several countries to include warning of hazards in laboratory methods. Subject to adjustment in this context and to any minor amendment coming from the Main Committee, these will be passed to the Secretariat E.B.C. on 1st August 1979 to arrange printing.

III. The Sub-Committee recommended that the Main Committee reviews safety aspects of all Analytica methods.

IV. The draft publication giving statistical data from the collaborative trials of the International Methods, which analyse for specific constituents, will be circulated to the Main Committee and, if approved, will be submitted for publication at the end of July.

V. Prof. Verzele's paper on H.P.L.C. and criticism of conductometric procedures was considered. Although the criticisms are scientifically correct, it was judged that they are not sufficient to invalidate the conductometric methods for commercial purposes. Hence, the amended procedure given in his paper will be compared collaboratively, with the current version in Analytica.

VI. Results for the analysis of the proposed E.B.C. Standard isomerised extract indicated that the material is inhomogeneous and therefore unsuitable. Dr. Pfenninger will try to obtain a supply of synthetic isohumulone to replace it.

VII. It was decided not to undertake collaborative studies on H.P.L.C. methods at present.

VIII. Results for the method for measuring Seed Content were judged sufficiently good for the adoption of the U.S.D.A. methods and an appropriate recommendation should be put to the Main Committee.

THE BREWING RESEARCH FOUNDATION

Research Establishment

From J.R. HUDSON, Ph.D., F.R.I.C.
Deputy Director

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Redhill, Surrey.
RH1 4HY

Tel: Nutfield Ridge (073 782) 2272
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19th September, 1979

Dear Gerald,

I enclose a copy of the paper which justifies the introduction of the new methods of hop analysis. This has been circulated to members of the Main Committee, but no one has suggested any alterations. Will you therefore arrange for its publication in the various Journals and appropriate languages?

I will send, in a few day's time, the copies of the new methods for Analytica which have now been agreed.

With kind regards,

Sincerely yours,

Ing.G.H.Ulenberg,
Secretary-General of the EBC,
c/o P.O.Box 510,
2380 BB ZOETERWOUDE,
The Netherlands.

ANALYSIS OF HOPS AND HOP PRODUCTS FOR α -ACIDS OR ISO- α -ACIDS

Submitted on behalf of the E.B.C. Analysis Committee

BY J.R. HUDSON

(Brewing Research Foundation, Nutfield, Redhill, England)

Two methods based on the resolution of mixtures of hop compounds by chromatography on Sephadex columns have been adopted by E.B.C. and A.S.B.C. as "International Methods".

Introduction

The Analysis Committee of the E.B.C. appointed J.R. Hudson, J. Maier, M. Moll, H. Pfenninger, I. Rosendal and M. Verzele as a Sub-Committee to recommend reference methods for the analysis of hops and hop products. Collaborative work has been carried out in conjunction with the corresponding Sub-Committee of the American Society of Brewing Chemists under the Chairmanship of Miss Gail Nickerson.

Rapid methods for the analysis of hops and hop extracts are given in *Analytica* and, despite occasional difficulties, serve for the valuation of hops and for the routine control of bitterness in brewing. However, the methods do not measure specifically the α -acids which are the major bittering constituents. Hitherto, no method of measuring the iso- α -acids in isomerized products has been included in *Analytica*. Hence, the collaborative studies were made on methods which separate specific groups of compounds prior to measurement.

Otter, Silvester and Tylor (1) introduced chromatography on QAE Sephadex A-25 for the resolution of mixtures of hop compounds. One modification of their technique by Anderegg, Schur and Pfenninger (2) is now recommended by both E.B.C. and A.S.B.C. on the basis of results from 13 laboratories shown in Table I and Fig. 1a and 1b. Another modification by Nickerson & Likens (3) has already been adopted by A.S.B.C. and following the results given in Table II and Fig. 2a and 2b has also been adopted by E.B.C. Both of these methods are therefore classified as International Methods.

The feature of difference between the two techniques is that in the Anderegg et al version, automated gradient elution is employed whereas in the Nickerson & Likens version stepwise gradient elution is employed.

TABLE I. Collaborative Analysis of Extracts by the Anderegg Schur and Pfenninger Procedure. (Two samples of each type were analysed.)

	Hop Extracts	Isomerized Extracts
Mean (all results) %	41.7	20.7
Sr	1.23	0.94
CVSr	<u>2.95</u>	<u>4.54</u>
Sb	1.44	1.81
CVSb	<u>3.45</u>	<u>8.74</u>
Sd	2.39	2.72
CVSd	<u>5.73</u>	<u>13.14</u>
No. of labs.	13	12
95% CR	3.01	2.31
F	3.75	8.34

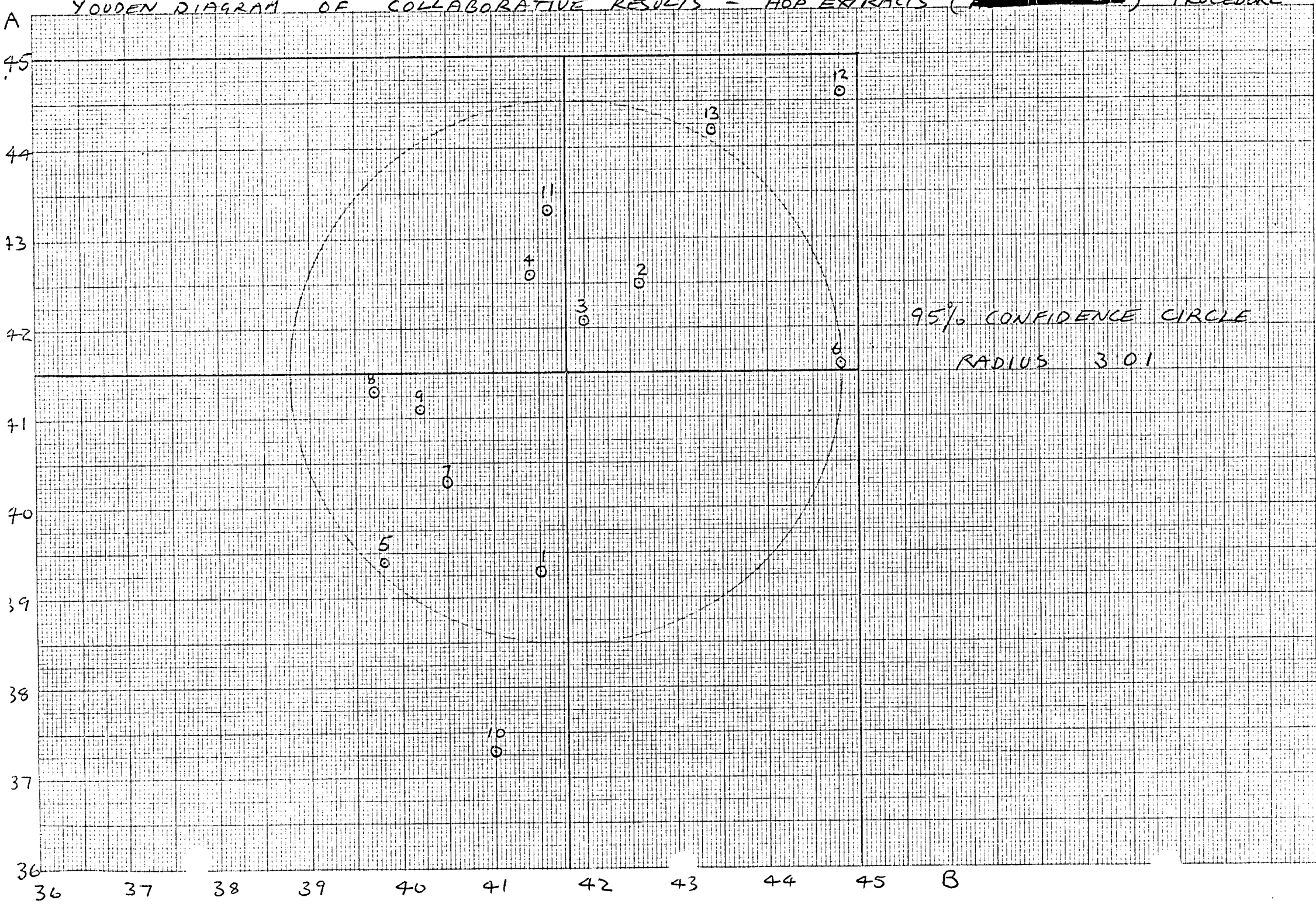
TABLE II. Collaborative Analysis of Extracts by the Nickerson & Likens Method. (Two samples of each type were analysed.)

	Hop Extracts	Isomerized Extracts
Mean (all results) %	30.2	21.8
Sr	1.04	0.88
CVSr	<u>3.44</u>	<u>4.04</u>
Sb	1.03	0.95
CVSb	<u>3.41</u>	<u>4.36</u>
Sd	1.79	1.61
CVSd	5.93	7.39
No. of labs.	14	13
95% CR	2.54	2.16
F	2.96	3.35

Fig 14

YOUDEN DIAGRAM OF COLLABORATIVE RESULTS - HOP EXTRACTS

ANDEREGG, SCHUR AND PFENNINGER
(~~XXXXXXXXXX~~) PROCEDURE



YODEN DIAGRAM OF COLLABORATIVE RESULTS - ISOMERISED EXTRACTS (ASBC METHOD)

A

26

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23

22

21

20

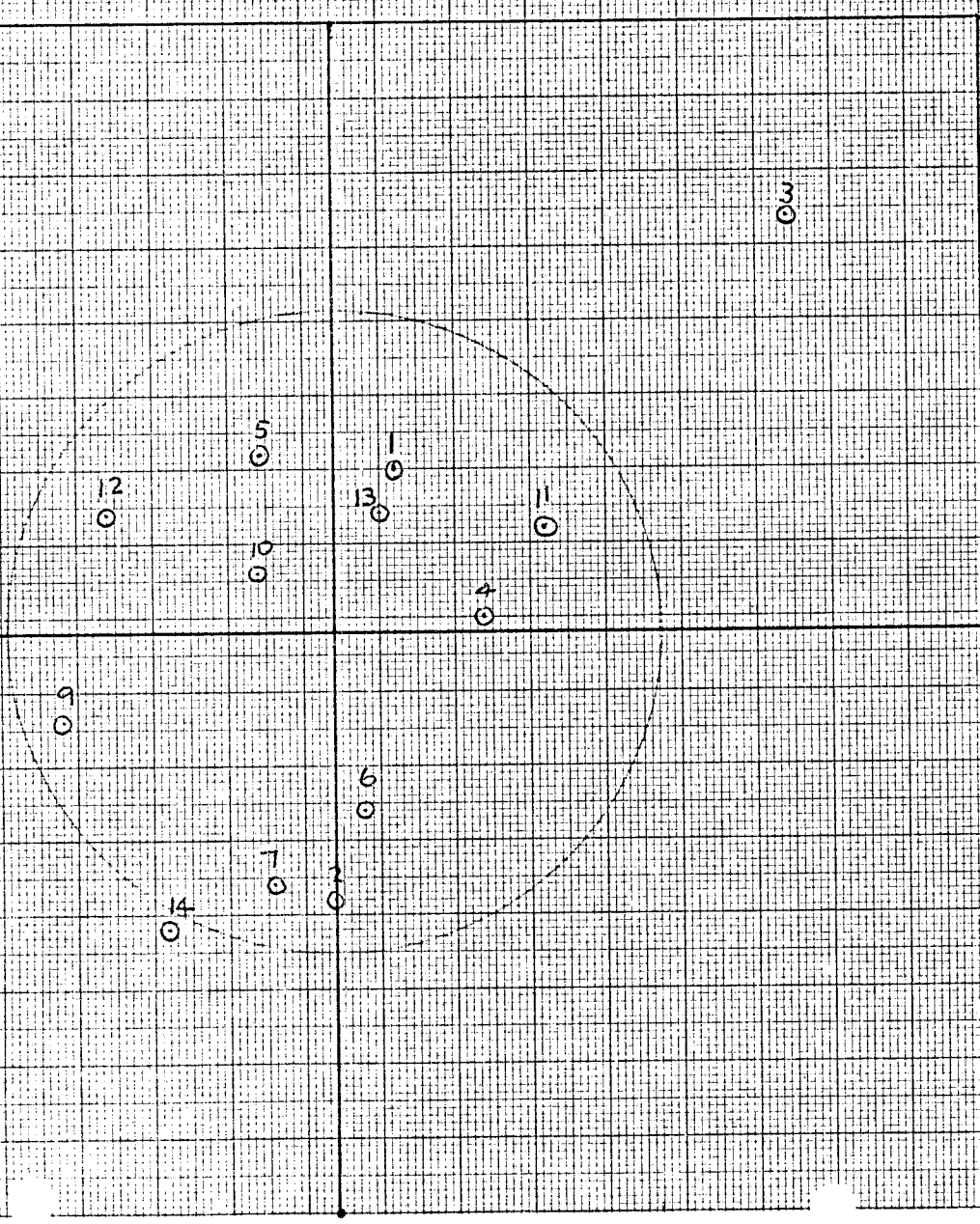
19

18

18 19 20 21 22 23 24 25 26

95% CONFIDENCE CIRCLE
RADIUS 2.16

B



0.9 L^u

YOUDEN DIAGRAM OF COLLABORATIVE RESULTS - HOP EXTRACTS (ASBC METHOD)

A

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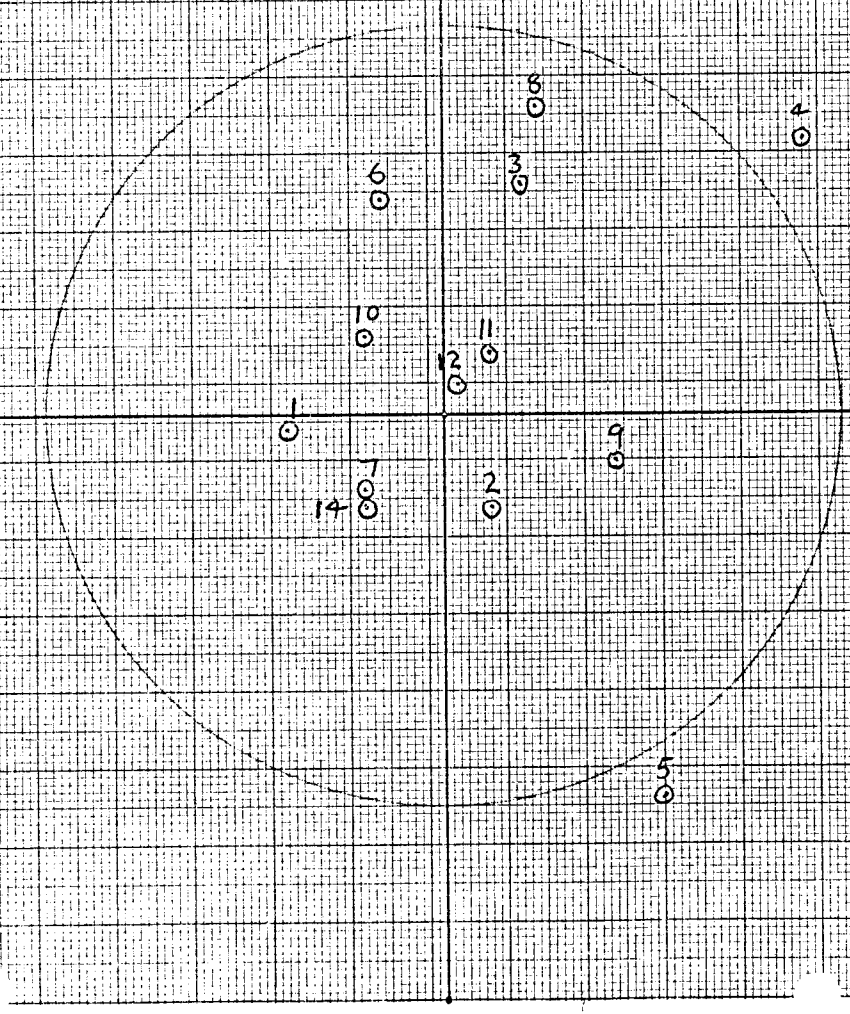
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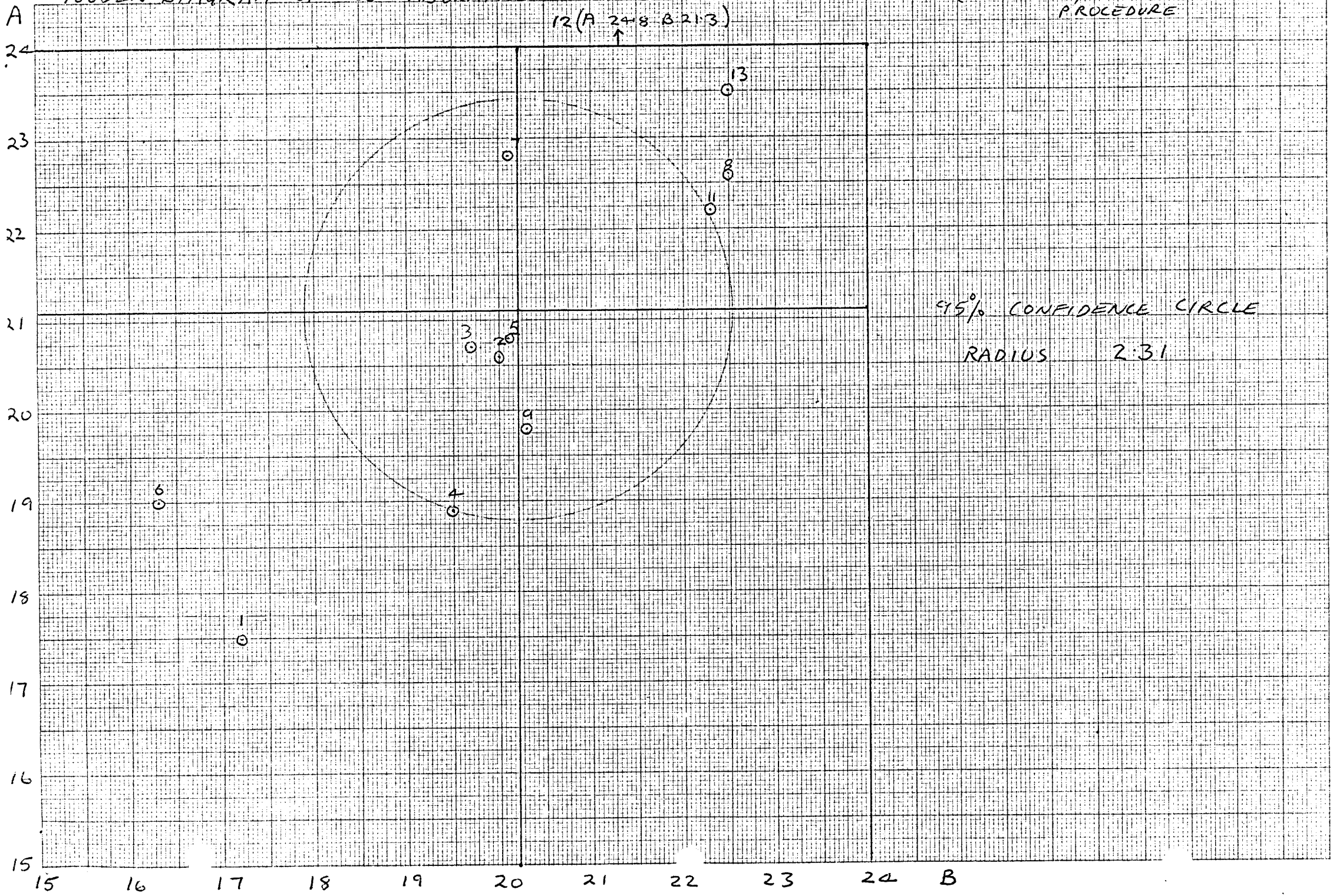
32

B



95% CONFIDENCE CIRCLE
RADIUS 2.54

Fig 1b
YOODEN DIAGRAM OF COLLABORATIVE RESULTS - ISOMERISED EXTRACTS (ANDEREGG, SCITUR AND PFENNINGER)
PROCEDURE



References

1. Otter, G.E., Silvester, D.J., & Taylor, L.,
J. Inst. Brew. 1972, 78, 57.
2. Anderegg, P., Schur, F., and Pfenninger, H.,
Schweiz., Brau. Rundsch. 1977, 88, 301.
3. Nickerson, G.B., and Likens S.T.
J. Amer. Soc. Brew. Chem. 1978, 36(1), 23.



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To the:

- Member Organisations
- Council Members
- Members of the Analysis Committee
- „ „ „ Barley Committee
- „ „ „ Biochemistry Group
- „ „ „ Microbiology Group
- „ „ „ Information & Documentation Group

of the European Brewery Convention

Our ref. : 46/80/U1/mvw

ZOETERWOUDE, 7th January 1980

Your ref.:

Please deal with only one subject per letter

Dear Ladies, Dear Sirs,

We have pleasure in sending you enclosed copies of the following, up-dated lists:

- Member Organisations
- Council Members
- Analysis Committee
- Barley Committee
- Chairmen of Committees and Groups
- Biochemistry Group
- Microbiology Group
- Information and Documentation Group

of the European Brewery Convention.

We request you to be so kind as to send any corrections and/or additional information to the Secretariat of the European Brewery Convention, P.O. Box 510, NL-2380 BB Zoeterwoude, The Netherlands.

With kind regards,
yours sincerely,

encls.

G.H. Ulenberg



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ANALYSIS OF HOPS AND HOP EXTRACTS

For some years, the EBC Secretariat have maintained a supply of hop extract which serves as an analytical standard for the determination of the "Conductometric Lead Value". A new batch, "5th EBC Standard Hop Extract" has been adopted following satisfactory collaborative analysis and will be available, free of charge, from 1st March 1980, onwards, from:

EBC Secretariat
P.O. Box 510
NL-2380 BB Zoeterwoude
The Netherlands

Analytical data are as follows:

5th EBC Standard Hop Extract

	<u>Lead Conductance Value %</u>
Average (from 9 laboratories)	42.5
Standard Deviation	1.16

Zoeterwoude, 4th February 1980

/s/

