THE VARIATIONS IN COLOUR OF LIQUID EXTRACT OF LIQUORICE B.P.

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From The British Drug Houses Ltd., London, N.1

Received July 1, 1949

IT is well known that variations in colour are found between batches of liquid extract of liquorice B.P. prepared from different samples of liquorice root.

Colour differences in liquid liquorice extracts are usually noticed in the dispensing of mixtures having the extracts as a constituent; the dilution obtained in such mixtures is often such as to bring it within what might be called the "critical" colour range. Variations in colour are not readily apparent until a dilution of 1 in 10 is reached, but differences can be detected in dispensing a number of common National Formulary mixtures. Thus Mistura Ammonii Chloridi contains a 15 minim dose and Mistura Ammoniae et Ipecacaunhae Composita a 10 minim dose of liquid extract of liquorice; these amounts represent dilutions of 1 in 16, and 1 in 24 respectively, and variations in the colour of the liquid extract of liquorice used will result in a different colour for the final dispensed mixtures, which may cause comment. In an endeavour to trace the reasons for these variations, details of the method of preparation have been examined, together with differences inherent in the drug and in the final product.

The British Pharmacopœia 1948 directs that unpeeled liquorice root, *Glycyrrhiza glabra* and other species of *Glycyrrhiza* shall be used in the preparation of liquid extract of liquorice. There are considerable differences in commercial samples of liquorice root and Wallis[1] gives figures of 15 to 27 per cent. for aqueous extractive, dried at 100°C.; the B.P. excludes samples of root with a water-soluble extractive below 20 per cent. Liquorice root of commerce is at present imported mainly from Anatolia, Syria or Iraq as ordinary or "natural" root; another type available is known as "cuttings" which are selected pieces of uniform

TABLE I

ANALYSIS OF COMMERCIAL SAMPLES OF LIQUORICE ROOT

Description of material	Moisture	Ash	Acid-insoluble ash	Water-soluble extractive (on material as received)
	per cent.	per cent.	per cent.	per cent.
Natural, unpeeled	8·3	4·4	0·4	20·5
Cuttings, unpeeled	8·6	5·1	0·6	31·6
Powdered decorticated*	7·0	3·9	0·1	41·2
Cuttings, unpeeled	9·1	6·9	0·6	35·3
Natural, unpeeled	10·7	5·4	0·6	25·1
Cuttings, unpeeled	8·5	5·2	0·2	33·1
Natural, unpeeled	8·4	7·6	0·9	25·9
Natural, unpeeled	7·0	5·1	0·3	21·0

* Described as "pulv. decort. elect."

size and diameter, the length of each piece being approximately 2 to 4 inches. A selection of samples of liquorice root obtained from different sources during recent years gave the analytical results shown in Table I and indicate the variations to be expected.

The figures are chiefly of interest in showing the wide variations in water-soluble extractive encountered in different samples of the crude drug; the remarkably high extractive in the single sample of commercial powdered decorticated root is also noteworthy. The method used for the determination of water-soluble extractive was similar to that described in the B.P. 1948.

Examination of the colouring matter

Representative samples of each of 6 lots of liquorice root, which complied with the requirements of the B.P. 1948, were reduced to a coarse powder and macerated with chloroform water (5 g. root to 100 ml.) for 24 hours, shaking frequently. The resulting product was filtered and aliquot portions of the filtrate were taken for determination of the water-soluble extractive and also for recording the colour in Lovibond units in a Lovibond Tintometer using a 1 cm. cell. The results obtained are given in Table II.

TABLE II

COLOUR VALUES OF SAMPLES OF LIQUORICE ROOT

Sample	Source and description of root	Water-soluble extractive	Lovibond Units using a 1 cm. cell			
			Yellow	Red	Blue	Neutral
		per cent.				
A.	Anatolian, natural	35·9	9·6	5·1	—	—
B.	Iraq, natural	26·9	10·1	6·2	—	0·1
C.	Syrian, natural	20·5	11·0	5·7	—	0·2
D.	Anatolian, cuttings	32·3	11·5	4·5	—	0·2
E.	Anatolian, cuttings	33·7	11·0	4·2	—	—
F.	Anatolian, cuttings	34·5	11·6	5·2	—	0·2

It can be seen from Table II that although the water-soluble extractive varied from 20·5 per cent. in sample C to 35·9 per cent. in sample A, the colour of the extracts did not vary appreciably; the composition and intensity of colour expressed as above were in fact relatively constant, despite the differences in origin and characteristics of the samples. Thus, under the conditions of experiment used, it can be concluded that the "depth" (composition and intensity) of colour extracted per unit weight of root appears to bear no relation to the percentage of water-soluble extractive obtainable from the material.

Usually most samples of extract of liquorice throw out a precipitate when added to acid solutions. For the preparation of liquorice extracts a dilute ammonia solution may be used in the process as, for example, in the preparation of liquorice root fluid extract, U.S.P. XIII, and Berg[2] used a menstruum containing ammonia for the extraction of liquorice by percolation. To study the effects on the resultant colour, further 5 g. quantities of the materials reported in Table II were macerated using a dilute (1 per cent. w/w) solution of ammonia in place of chloro-

form water. The proportion of extractive obtained was determined and the colour examined as before. The results obtained are given in Table III.

TABLE III

EXTRACTIVES AND COLOUR OF EXTRACTS OBTAINED WITH A DILUTE SOLUTION OF AMMONIA

Sample of Liquorice Root (see Table II)	Dilute Ammonia-soluble extractive	Lovibond Units using a 0·25 cm. cell			
		Yellow	Red	Blue	Neutral
	per cent.				
A.	34·5	12·3	3·8	—	0·1
B.	29·5	10·0	3·5	—	—
C.	21·8	13·4	5·5	—	—
D.	33·5	13·2	5·0	—	—
E.	36·4	19·0	3·3	—	0·2
F.	34·5	18·2	4·4	—	0·2

The use of dilute solution of ammonia did not increase significantly the total percentage of extractive, as can be seen by comparison of the extractive figures for the respective samples given in Table II and Table III.

It should be noted, however, having regard to the smaller cell width used, that the intensity of colour of the extract as expressed had been very appreciably increased. The question arises, therefore, whether the increased intensity of colour results from the extraction of additional coloured materials by the alkaline solution or is simply due to a darkening or " indicator " effect at alkaline *p*H values.

A sample of aqueous extract (5 g. of liquorice root, 100 ml. of water) was therefore adjusted to various *p*H values by the addition of small amounts of either sodium hydroxide solution or hydrochloric acid and the colour examined (see Table IV). The *p*H value of the extracts before addition of acid or alkali was approximately 6·5.

TABLE IV

EFFECT OF *p*H ON THE COLOUR OF AN AQUEOUS EXTRACT OF LIQUORICE ROOT

*p*H Value of extract after addition of acid or alkali	Lovibond Units using a 0·25 cm. cell			
	Yellow	Red	Blue	Neutral
2·0		Cloudy solutions		
4·0				
6·5	2·3	0·7	—	—
7·0	4·6	1·0	—	—
7·5	4·9	1·0	—	0·1
8·5	6·0	1·6	—	0·1
9·0	9·0	1·9	—	0·2
9·5	13·9	2·4	—	0·3
10·0	16·0	3·0	—	0·1

In acid solutions (low *p*H values) precipitation occurred which interfered with the examination of the colour of the liquid. It can be seen, however, that as the *p*H increased and especially on the alkaline side the extracts became considerably darker, both yellow and red components increasing in intensity. The colour obtained at each *p*H value was stable,

however, and showed no significant change over a period of two days. It was noted incidentally that on the addition of alkali a buffering effect occurred between *p*H 8·5 and 9·0 and the addition of a relatively large quantity of sodium hydroxide solution was required to increase the *p*H above 8·5.

Because of the difficulty of measuring directly the colour of the extracts at low *p*H values, the effect of acid and alkali on the colour isolated chromatographically was examined. A sample of aqueous extract of liquorice root was passed through a column of alumina when the colour was adsorbed. Washing down the column with quantities of water then produced a dilute aqueous solution of the coloured materials of the extract substantially free from other water-soluble matter. Aliquot quantities of this solution were then adjusted to various *p*H values and the colour examined. The results obtained are given in Table V.

It can be seen that there is a definite " indicator effect " obtainable with the colouring matter from extract of liquorice, the colour being much lighter in acid and darker in alkaline solution. This effect was found to be reversible. Addition of alkali to an acidified solution quantitatively restored the colour, and addition of acid to an alkaline solution diminished the colour, which could be restored on making alkaline once more.

TABLE V

EFFECT OF *p*H ON THE COLOUR OF AN AQUEOUS SOLUTION OF THE COLOURING MATTER ISOLATED FROM AN EXTRACT OF LIQUORICE ROOT

*p*H	Lovibond Units using a 0·25 cm. cell			
	Yellow	Red	Blue	Neutral
2·0	0·4	—	—	—
4·0	0·8	—	—	—
6·5	1·0	—	—	—
8·0	1·1	—	—	0·2
9·0	2·4	0·1	—	0·1
10·0	2·7	—	—	—
10·5	2·8	—	—	—

It will be realised from the results reported that, according to the conditions of preparation and use of liquid extract of liquorice B.P. so there can result variations in the colour of the products.

After evaporation according to the official instructions a *p*H slightly on the acid side is usually obtained, but the variation of acidity encountered is not such as to result in appreciable variations in colour due to the indicator effect referred to above. Since, however, the B.P. instructs " evaporate until the weight per ml. of the liquid at 20°C. is 1·198 " it follows that the standardisation of the liquid extract on total water-soluble extractive, though entirely logical for such a product, may cause relatively appreciable differences in colour between extracts prepared from samples of root showing a relatively high, and from those showing a relatively low, water-soluble extractive. Thus, for example, a sample of liquorice root giving a high water-soluble extractive will give a comparatively large volume of final product and since, as was shown in

Table II, the amount of coloured materials extracted is independent of the water-soluble extractive of the root, the resulting preparation will be relatively light in colour. Using water-soluble extractive as a criterion of quality of liquorice root it follows that a good quality root may, in fact, result in an official extract relatively light in colour. The yields and final colours of a number of prepared samples of liquid extract of liquorice B.P. were therefore noted and these are given in Table VI; the recorded yields were obtained using B.P. quantities and the Lovibond Tintometer readings are for 1 in 100 aqueous dilutions of the liquid extract. These results show that a high yield was associated in these examples with a relatively low colour intensity. Having regard, however, to the variations encountered between laboratory and industrial methods of evaporation and to lack of knowledge of the influence of heat on the constituents of the extract responsible for its colour, it would not be justifiable to expect such a relationship to be applicable generally.

TABLE VI

CORRELATION BETWEEN YIELD AND COLOUR FOR SAMPLES OF LIQUID EXTRACT OF LIQUORICE B.P.

Example	Yield	Lovibond Units using a 1 cm. cell			
		Yellow	Red	Blue	Neutral
	ml. per 1000 g. of root				
1	481	14·1	1·7	—	—
2	678	7·0	0·9	—	—
3	566	10·5	1·3	—	—
4	655	7·0	0·9	—	—
5	368	14·2	1·8	—	—
6	671	7·5	0·9	—	—
7	716	5·4	0·7	—	—
8	569	6·5	0·8	—	—

It is interesting to note that, while the British Pharmacopœia 1948 adopts the more logical procedure of standardisation on solid content, thus leading to the possibility of variations in colour according to quality of root used, the method of the United States Pharmacopœia XIII will lead to the production of extracts varying in solid content according to the water-soluble extractive of root used, but the extracts obtained are likely to be relatively constant in colour.

The colour measurements given in Table VI are, as mentioned, those obtained in aqueous dilutions only. The colour in dispensed mixtures will, of course, differ according to a number of factors as well as the *pH* of the solution; for example, the nature and concentration of the electrolytes present may also cause alterations in colour.

Certain Aspects arising during Manufacture. In the preparation of the liquid extract the B.P. directs that unpeeled liquorice root, in coarse powder, shall be exhausted by percolation with chloroform water, the percolate boiled and set aside for not less than 12 hours, the clear liquid decanted and the remainder filtered from the relatively light-coloured sludge present in the latter being rejected. This sludge is not sufficiently coloured, however, to affect the colour of the final preparation. It is not

advisable to set aside the preparation for any length of time at this stage, owing to the likelihood of fermentation. In practice on the manufacturing scale, evaporation may be done under reduced pressure so that changes in the colour of the product due to overheating are less likely to occur.

As it was thought possible that differences in the time for which the preparation is allowed to stand before filtration (the B.P. states " not less than forty-eight hours ") might cause slight differences in the colour of the final preparation, the effect of time of standing was examined. After standing for three weeks a small heavier layer collected, giving the following comparative results (1 in 100 dilution, Lovibond Units):

Extract immediately after preparation:	Yellow 8·0; Red 1·0
Extract (lower layer) after standing for three weeks	Yellow 10·6; Red 1·3

Although, as can be seen, the lower layer was relatively more intensely coloured, the actual volume of this layer was small in comparison with the bulk of the preparation and the amount, later rejected, did not appreciably affect the colour of the bulk of the extract.

Summary

1. A study has been made of the colouring matter extracted from liquorice root and of some of the possible causes of variation in colour of liquid extract of liquorice B.P.

2. It has been shown that the colour variation occurring in samples of extract prepared according to the official instructions may result from the standardisation of the preparation on its content of total water-soluble extractive.

The effect of changes in *p*H value on the colour of liquid extract of liquorice has also been investigated.

The authors wish to thank Miss S. M. Stokes for assistance in carrying out the practical work, and the Directors of The British Drug Houses, Ltd., for permission to publish this paper.

References

1. Wallis, *Textbook of Pharmacognosy*, 1946, Churchill, 334.
2. Berg, *J. Amer. pharm. Ass., Sci. Ed.*, 1924, **13**, 814.

DISCUSSION

The paper was read by Mr. J. H. Oakley.

The Chairman said that it was interesting to note the increasing use of chromatography, which was coming into a large number of Conference papers.

Dr. J. M. Rowson (London) remarked that nothing seemed to be known about the nature of the colouring matter present in liquorice root and the standard textbooks made virtually no reference to it. He wondered whether the authors had any idea of the chemical nature of