# THE SPECTROPHOTOMETRIC DETERMINATION OF RUTIN AND QUERCETIN IN MIXTURES

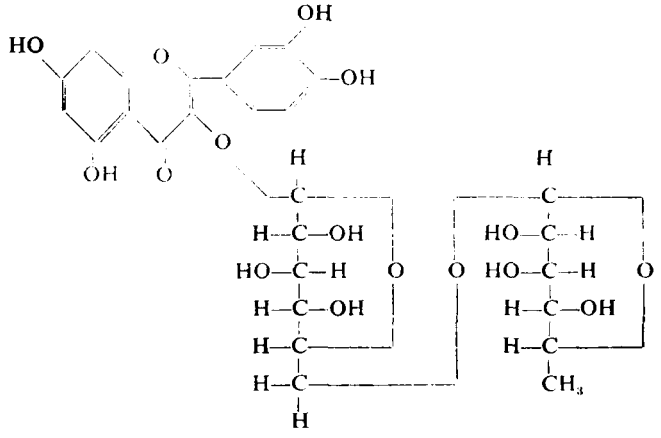
By R. V. Swann

*From the Control Laboratory, Allen and Hanburys, Ltd.*

Received January 20, 1949

Rutin is a flavonol glycoside which is derived from quercetin by condensation of the sugar portion of the molecule with the phenolic hydroxyl group at position 3 of quercetin. On hydrolysis with dilute acid, rutin yields quercetin, rhamnose and glucose in equimolecular proportions.

Rutin has the following formula:



Tentative methods for the determination of rutin and quercetin have been suggested by Porter, Brice, Couch and Copley[1], and their methods have been modified by the Research Division of Penick and Co., New York.[2] The methods of Porter *et al.* are based on the determination of $E_{362\cdot5}$* and $E_{375}$ for rutin and quercetin.

Penick and Co. state that if the ratio $\frac{E_{375}}{E_{362\cdot5}} = 0\cdot875 \pm 0\cdot004$ the quercetin content is reported as being less than 1 per cent., and the percentage of rutin is given by the relation $\frac{E_{362\cdot5} \times 100}{325\cdot5}$ where $325\cdot5 = E_{362\cdot5}$ for pure anhydrous rutin. It will later be shown that $\frac{E_{375}}{E_{362\cdot5}}$ is approximately 0·876 for rutin containing 1 per cent. of quercetin and that the contribution of 1 per cent. of quercetin to the gross absorption is approximately equivalent to that given by 2 per cent. of rutin. Thus in the case of rutin containing 1 per cent. of quercetin the rutin content given by the ratio $\frac{E_{362\cdot5} \times 100}{325\cdot5}$ will be 2 per cent. in excess of the true

*Throughout the paper $E_x$ is used to indicate $E\ \frac{1\ \text{per cent}}{1\ \text{cm.}}$ at wave length x mμ

value. If the ratio $\frac{E_{375}}{E_{362\cdot5}}$ is greater than 0·879, Penick and Co. calculate the rutin content from the formula.

$$\text{Rutin per cent.} = 1\cdot4722\ E_{362\cdot5} - 1\cdot3324\ E_{375}$$

and Quercetin per cent $= -0\cdot5406\ E_{362\cdot5} + 0\cdot6183\ E_{375}$

Further, small differences, within the limits of experimental error in the values of $E_{362\cdot5}$ and $E_{375}$ for rutin and quercetin will alter the formulæ with the result that the rutin content as given by the formula is only likely to be correct within $\pm$ 0·5 per cent. under optimum conditions.

Both Penick and Co. and Porter *et al.* previously dry the sample at 125°C. for 4 and 16 hours respectively in high vacuum before carrying out the assay. If the assay is to be applied to commercial samples such a procedure would necessitate a moisture determination carried out under the same conditions, and the time required to carry out an assay would be considerable. For this reason and the fact that no claims have been made to estimate less than 1 per cent. of quercetin in rutin-quercetin mixtures it was decided to undertake a systematic study of the problem.

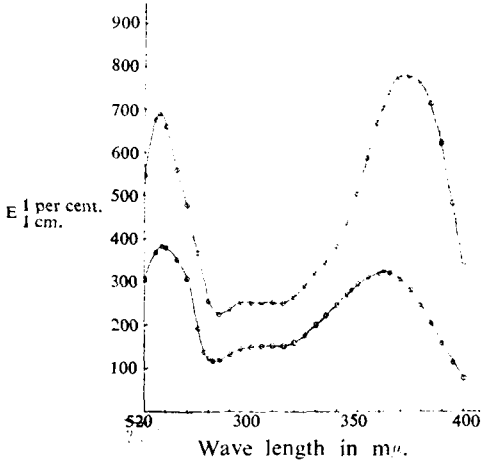



FIG. 1. Absorption spectra

Upper graph—quercetin. Lower graph—rutin

*Absorption Spectra of Rutin and Quercetin.* When dissolved in alcohol (95 per cent.) containing 1 per cent. of 0·02N acetic acid both rutin and quercetin obey Beer's and Lambert's laws. As shown in Figure 1 rutin exhibits absorption maxima at 259 mμ and 362·5 mμ and quercetin exhibits maxima at 257 mμ and 375 mμ. The maxima at 362·5 mμ and 375 mμ are suitable for the determination of rutin and quercetin in mixtures.

*Choice of Solvent.* It was found that for rutin $E_{362\cdot5}$ increased with increasing alcohol concentration without marked change in the wave-

TABLE I

EFFECT OF INCREASING THE CONCENTRATION OF ALCOHOL ON WAVE LENGTH OF MAXIMUM ABSORPTION

| Alcohol Concentration | $E_{362\cdot5}$ | Alcohol Concentration | $E_{362\cdot5}$ |
|---|---|---|---|
| 50 per cent. ... ... | 277 | 90 per cent. ... ... | 292 |
| 60 ,, ... ... | 281 | 95 per cent. ... ... | 300 |
| 70 ,, ... ... | 285 | 95+1 per cent. of 0·02N acetic acid ... ... | 304 |
| 80 ,, ... ... | 288 | | |

length of maximum absorption. The results in Table I were obtained on a commercial sample.

The use of alcohol (95 per cent.) permits increased accuracy in the determination, since the solubility of rutin in acid alcohol (95 per cent.) is very much much greater than in alcohol (50 per cent.). Acetic acid (0·02N) is added to maintain the final dilution on the acid side of neutrality, since mention is made in the literature of the capacity of rutin to form metallic salts.[3,4]

*Hydration of Rutin.* If samples of rutin previously dried to constant weight at 110°C. are assayed, the maximum value of $E_{362\cdot5}$ which can be obtained for a sample which is completely soluble in acid alcohol (95 per cent.) is approximately 300. After drying samples at 110°C. in a vacuum below 1 mm. Hg. pressure over phosphorus pentoxide for 2 hours, and assaying the dried sample, it is found that the value of $E_{362\cdot5}$ increases to approximately 325. This corresponds to a loss of 7·7 per cent. of moisture on drying. Rutin is known to exist as the trihydrate and this contains approximately 8·1 per cent. of water of crystallisation. Thus, drying at 110°C. in a high vacuum for 2 hours removes all the water of crystallisation. From the above it seems likely that commercial samples of rutin will consist essentially of rutin trihydrate plus hygroscopic moisture. This has been confirmed for samples so far examined.

*Preparation of Pure Rutin.* A sample of crude rutin prepared by extraction from buckwheat was purified as follows:

A. The sample was dissolved in alcohol, the least possible quantity of alcohol being used, and the rutin was then reprecipitated by the addition of sufficient water to reduce the alcohol concentration below 10 per cent. After standing in a refrigerator overnight, the rutin was filtered and washed with water.

B. The purified rutin from section A was dissolved in the least possible quantity of alcohol (75 per cent.), warming to effect solution. The solution was cooled, and brown material, which was precipitated on cooling, was filtered off. The alcohol was then distilled off until the point of incipient crystallisation was reached. The solution was allowed to cool, the rutin filtered, washed with small amounts of alcohol (75 per cent.) and dried at 110°C.

C. The filtered rutin was then dissolved in boiling 99 per cent. *iso*propyl

TABLE II

SPECTROPHOTOMETRIC DATA FOR SAMPLES OF RUTIN AND QUERCETIN DRIED AT 110°C. IN A HIGH VACUUM

| | $E_{347}$ | $E_{362\cdot5}$ | $E_{375}$ |
|---|---|---|---|
| *Rutin* | | | |
| Original sample ... ... ... | 279·4 , 277·8 | 321·0 , 321·8 | 280·0 , 280·7 |
| Sample A ... ... ... ... | 282·5 , 282·8 | 325·2 , 325·5 | 284·9 , 283·0 |
| Sample B ... ... ... ... | 281·4 , 282·6 | 324·9 , 325·2 | 282·4 , 283·2 |
| Sample C ... ... ... ... | 281·2 , 281·0 | 325·2 , 324·5 | 283·1 , 283·0 |
| *Quercetin* | | | |
| Sample D ... ... ... ... | —— | 695 , 693 | 774 , 768 |
| Sample E ... ... ... ... | 460 | 705 | 786 |
| Sample F ... ... ... ... | 458 | 702 | 780 |

The values of $E_{347}$, $E_{362\cdot5}$ and $E_{375}$ for samples C and F did not alter with subsequent treatment.

alcohol, the solution was concentrated, cooled to room temperature, filtered and poured into 10 volumes of hot water. When precipitation was complete the rutin was filtered off.

*Preparation of Pure Quercetin.* D. Crude quercetin was prepared by refluxing purified rutin with 1 per cent. hydrochloric acid (100 ml./g. of rutin). The quercetin was filtered off and washed with cold water. The crude quercetin was twice crystallised from alcohol (80 per cent.). Samples E and F were taken from the first and second recrystallisations respectively and sample F melted at 313°C. Samples A. B. C. D. E and F were dried at 110°C. in a high vacuum and examined spectrophotometrically with the results given in Table II.

*Assay of Rutin and Quercetin in Mixtures.* According to Vierordt,[5] two components of a mixture may be assayed by means of the following formulæ, if values of E for both substances and for the mixture are known for two selected wavelengths.

In the present case

$$\text{Percentage of rutin} = \left(\frac{c_0b_1 - c_1b_0}{a_0b_1 - a_1b_0}\right) \times 100$$

$$\text{Percentage of quercetin} = \left(\frac{c_0a_1 - c_1a_0}{b_0a_1 - b_1a_0}\right) \times 100$$

where $a_0$ = $E_{362\cdot5}$ pure anhydrous rutin.
$a_1$ = $E_{375}$ pure anhydrous rutin.
$b_0$ = $E_{362\cdot5}$ pure quercetin.
$b_1$ = $E_{375}$ pure quercetin.
$c_0$ = $E_{362\cdot5}$ mixture.
$c_1$ = $E_{375}$ mixture.

The values of $a_0$, $a_1$, $b_0$ and $b_1$ have been determined experimentally and have been assigned the following values:—

$$a_0 = 325,\ a_1 = 283,\ b_0 = 702,\ b_1 = 780.$$

The equations then become

percentage of rutin = $1\cdot4224\ E_{362\cdot5} - 1\cdot2802\ E_{375}$
percentage of quercetin = $-\ 0\cdot5161\ E_{362\cdot5} + 0\cdot5927\ E_{375}$

The latest formulæ published by Penick and Co. are

percentage of rutin = $1\cdot4722\ E_{362\cdot5} - 1\cdot3324\ E_{375}$
percentage of quercetin = $-\ 0\cdot5406\ E_{362\cdot5} + 0\cdot6183\ E_{375}$

The results in Table III were given on mixtures of known composition, by the Penick formulæ, the formulæ derived above, and by a graphical method which will be described later in the paper.

From Table III it will be seen that in the case of the Penick formula, the percentage of quercetin is correct within the limits of experimental error to be expected in such a determination, whilst the rutin results with one exception are about 1 per cent. high. In the case of the derived formula, the percentage of rutin is approximately correct, whilst the quercetin results are about 0·5 per cent. high.

TABLE III

COMPARISON OF THE RESULTS OBTAINED BY THREE METHODS FOR MIXTURES OF KNOWN COMPOSITION

| Theoretical | | Penick Formula | | Derived Formula | | Graph | |
|---|---|---|---|---|---|---|---|
| Rutin per cent. | Quercetin per cent. | Rutin per cent. | Quercetin per cent. | Rutin per cent. | Quercetin per cent. | Rutin per cent. | Quercetin per cent. |
| 99·03 | 0·97 | 101·20 | 0·72 | 100·00 | 1·40 | 99·25 | 0·75 |
| 98·60 | 1·40 | 99·67 | 1·58 | 98·40 | 2·20 | 98·60 | 1·40 |
| 98·08 | 1·92 | 98·36 | 1·61 | 99·90 | 1·98 | 98·05 | 1·95 |
| 97·52 | 2·48 | 98·55 | 2·62 | 97·08 | 3·20 | 97·30 | 2·70 |
| 97·14 | 2·86 | 98·37 | 3·00 | 97·23 | 3·60 | 96·40 | 3·60 |
| 96·59 | 3·41 | 97·68 | 3·47 | 96·58 | 4·00 | 96·30 | 3·70 |
| 96·23 | 3·77 | 97·52 | 3·79 | 96·43 | 4·36 | 96·00 | 4·00 |
| 95·69 | 4·31 | 96·54 | 4·33 | 95·59 | 4·88 | 95·60 | 4·40 |
| 95·53 | 4·67 | 96·66 | 4·54 | 95·62 | 5·08 | 95·30 | 4·70 |

The mixtures were made from purified rutin and quercetin previously dried at 110° C. for 2 hours in a high vacuum.

Since the ratio $\frac{E_{347}}{E_{375}}$ is a measure of the quercetin content, it was decided to attempt to use the ratio to measure the quercetin content and hence the rutin content by difference or calculation. Table IV has been constructed from the data for rutin and quercetin.

TABLE IV

MEASUREMENT OF RUTIN CONTENT BY THE RATIO $\frac{E_{347}}{E_{375}}$

| Rutin per cent. | Quercetin per cent. | $\frac{E_{347}}{E_{375}}$ | $\frac{E_{375}}{E_{362\cdot5}}$ |
|---|---|---|---|
| 100 | 0 | 0·9936 | 0·871 |
| 99 | 1 | 0·9828 | 0·876 |
| 98 | 2 | 0·9722 | 0·881 |
| 97 | 3 | 0·9618 | 0·886 |
| 96 | 4 | 0·9520 | 0·891 |
| 95 | 5 | 0·9419 | 0·895 |
| 94 | 6 | 0·9330 | 0·900 |

It will be seen that the ratio $\frac{E_{347}}{E_{375}}$ changes more rapidly with the increased amounts of quercetin than the ratio $\frac{E_{375}}{E_{362\cdot5}}$

The ratio $\frac{E_{347}}{E_{375}}$ has been determined for the mixtures in Table III, and the percentage of quercetin read off from the graph plotted from the data in Table IV. It will be seen that in nearly every case the percentages of rutin and quercetin agree with the theoretical values $\pm$ 0·2 per cent. This method is not applicable to the rapid assay of commercial samples, as it would necessitate drying to constant weight in high vacuum at 110°C. However, as the graph will indicate the relative amounts of rutin and quercetin, by means of a formula, samples may be assayed without previous drying.

The following method is proposed for the routine assay of a rutin-quercetin mixture.

Weigh 50 mg. and dissolve in ethyl alcohol (95 per cent.), using 50-ml. graduated flasks. Dilute suitably to give a density reading of 0·4 to 0·6 when the wave length scale of the Beckman spectrophotometer is set at 362·5 mμ. Add 0·5 ml. of 0·02N acetic acid to the last dilution and compare the optical density of this solution with that of a solution of ethyl alcohol (95 per cent.), containing a similar amount of acetic acid, using 1 cm. cells, a tungsten lamp and a Corning No. 9863 Red-Purple Corex A filter.

Calculate $E_{347}$, $E_{362\cdot5}$, $E_{375}$ and the ratio $\frac{E_{347}}{E_{375}}$.

From Table IV or a prepared graph read off the composition of the mixture corresponding to the ratio.

If x = percentage of quercetin read on graph, then rutin : quercetin $= 100 - x : x$ or quercetin $= \left(\frac{x}{100 - x}\right)$ rutin.

Let the actual rutin content of sample be y per cent. anhydrous rutin, then quercetin content will be $\left(\frac{x}{100 - x}\right)$ y.

For a mixture $E_{362\cdot5} = 3\cdot25\ y + 7\ y \left(\frac{x}{100 - x}\right)$

Here x and $E_{362\cdot5}$ are known, so y, the anhydrous rutin content of the sample may be calculated.

From this the quercetin is given by $\left(\frac{x}{100 - x}\right)$ y

percentage of anhydrous rutin x 1·088 = percentage of rutin trihydrate.

It is important to ascertain that the maximum absorption does not lie on the ultra-violet side of 362·5 mμ. If the maximum is at a shorter wave length than 362·5 mμ, then the determination will be rendered inaccurate by the presence of other absorbing substances. None of the methods which have been proposed for the assay of rutin is directly applicable if there are considerable amounts of chlorophyll and red pigment present. Chlorophyll and red pigment may be tested for by the extraction of the sample with ether. If chlorophyll and/or red pigment are present, the ether will become coloured. Pure rutin is insoluble in ether. The above method may be applied after the chlorophyll and red pigment have been extracted. Porter *et al*[2] have given a method

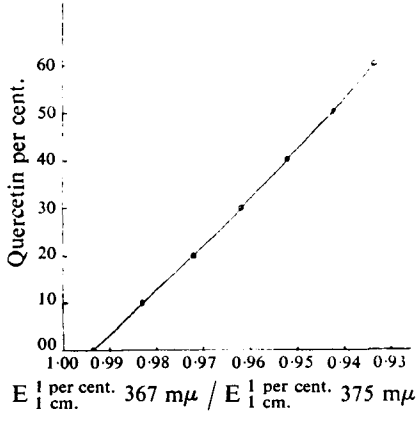



FIG. 2. Percentage of quercetin in mixtures of anhydrous rutin and quercetin.

for the quantitative determination of chlorophyll and red pigment in rutin.

### Assay of Rutin in Tablets

Determine the mean weight of 20 tablets. Powder and mix well. Weigh 50 mg. and dissolve in 80 ml. of ethyl alcohol (95 per cent.), warming to effect solution. Filter and make up to volume with ethyl alcohol (95 per cent.) in a 100-ml. graduated flask. Dilute suitably for the spectrophotometer, adding 1 per cent. v/v of 0·02N acetic acid to the final dilution. Then proceed as instructed for the assay of rutin and quercetin.

$$\text{Mean weight per tablet in mg.} \times \frac{\text{percentage of anhydrous rutin}}{100} = \text{mg. of anhydrous rutin per tablet.}$$

The above assay has been found satisfactory for tablets containing as excipients lactose, starch and gum acacia.

The spectrophotometric measurements in this investigation were carried out on a Beckman Quartz Spectrophotometer calibrated on the mercury lines of wave length 4047Å, 3650Å, 3341Å, and 3132Å.

### Summary

1. The ultra-violet absorptions of rutin and quercetin have been investigated.
2. A rapid method has been developed for the accurate determination of the minor constituent of binary mixtures of rutin and quercetin, and a formula derived for the correction of the gross absorption for the absorption due to the minor constituent.
3. A comparison has been made between American methods of assay and the method in use in this laboratory.

The author wishes to express his thanks to the Directors of Allen and Hanburys, Ltd., for permission to publish this paper, to Dr. N. Evers for helpful criticism, and to Mr. W. Smith for providing laboratory facilities.

### References

1. Porter, Brice, Copley and Couch *Tentative Spectrophotometric Method for the Determination of Rutin in Various Preparations.* Eastern Regional Research Laboratory, U.S. Department of Agriculture.

2. *Rutin—Provisional Standards and Specifications.* Research Division of S. B Penick and Company, New York, 1948.

3. Schunck, *J. chem. Soc.*, 1888, 262.

4. Tiemann. *Arch. Pharm.*, 1903, **241**, 289.

5. Vierordt. *Die Anwendung des Spectralapparates zur Photometrie der Absorptionsspectren und zur quantitativen chemischen Analyse.* Tübingen, 1873, 51.