Quantitative Analysis of Flavonoids

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Some flavonoids, quercitrin, quercetin, and rutin are analyzed quantitatively using zirconium chloride or diphenylboric acid β-aminoethyl ester as a color reagent. These methods are suitable for the quantitative analysis of flavonoids contained in vegetable crude drugs and pharmaceutical preparations.

THE QUANTITATIVE analysis of rutin and its preparations by the determination of the ultraviolet absorption (UV) has been well known (1–4). In the present investigation on the quantitative analysis of rutin (I), quercitrin (II), and quercetin (III), their preparations were investigated. Studies on the quantitative analysis were restricted to colorimetry, and zirconium chloride (*A*) and β-aminoethyl diphenylborate (*B*) (5) were used as the color reagents.

The relative concentration of the colored complex seemed to depend on the pH of the reaction medium. The pH profile for the appearance of the colored complex was determined in acetic acid-sodium acetate buffer (Figs. 1 and 2). The buffer solutions

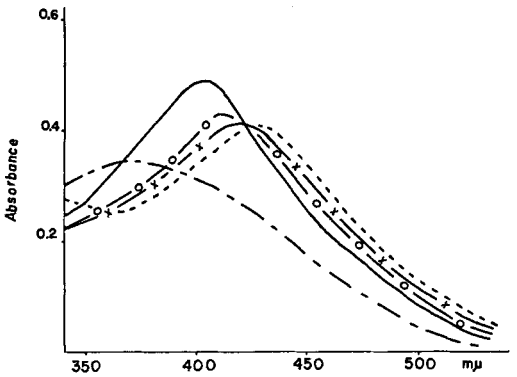

Fig. 1—UV spectra of flavones colored by method A *(rutin) in various buffer solutions. Key:* — — —, *2* M *AcOH;* ———, *2* M *AcOH–2* M *AcONa (3:1);* O, *2* M *AcOH–2* M *AcONa (1:1);* X, *2* M *AcOH–2* M *AcONa (1:3);* – – – –, *2* M *AcONa.*

suitable for method *A* were acetic acid–sodium acetate (3:1) and for method *B*, sodium acetate. The characteristic reactions of these flavones produced a yellow color with method *A* ($\lambda_{max.}$: I, 400 mμ; II, 402 mμ; III, 468 mμ) and method *B* ($\lambda_{max.}$: I, 404 mμ; II, 394 mμ; III, 406 mμ). Calibration curves (Fig. 3) for complexes of I, II, and III by both methods *A* and *B* suggested the possibility of quantitative analysis.

The quantitative analytical data for pharmaceutical preparations are summarized in Table I. Some of the crude drugs analyzed as quercitrin (No. 1) or quercetin (No. 2), and simple preparations of flavones (I, II) and containing ascorbic acid (Nos. 3~6) also gave good quantitative analytical data. The data also revealed that the presence of hesperidin, vitamin K, and carbazochrome showed no interference. On the other hand, preparations containing methylhesperidin, vitamin K_4 diacetate, and/or extracts of coptis, rheum, and scutellaria showed rather a large observational error.

EXPERIMENTAL

Sample solutions were prepared by dissolving 5 mg. each of the materials in 50 ml. of methanol in a

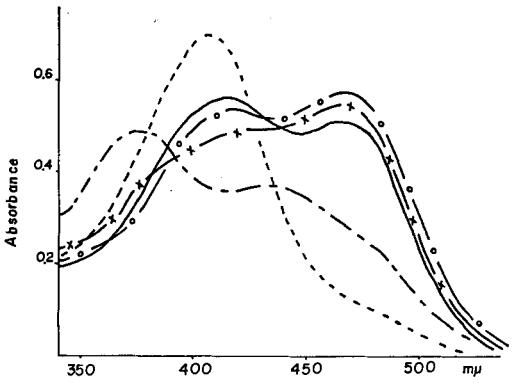

Fig. 2—UV spectra of flavones colored by method B *(quercetin) in various buffer solutions. Key:* — — —, *2* M *AcOH;* ———, *2* M *AcOH–2* M *AcONa (3:1);* O *2* M *AcOH–2* M *AcONa (1:1);* X, *2* M *AcOH–2* M *AcONa (1:3);* – – – –, *2* M *AcONa.*

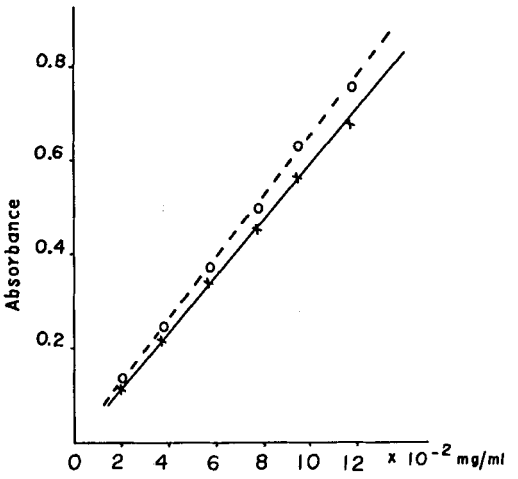

Fig. 3—Calibration curves of rutin by methods A *and* B. *Key: X, method* A; O, *method* B.

Received July 13, 1967, from the College of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo, Japan.
Accepted for publication November 15, 1967.

TABLE I—QUANTITATIVE ANALYSES OF RUTIN IN PHARMACEUTICAL PREPARATIONS

No.	Preparation (Components)	% of Theory—Method *A*	% of Theory—Method *B*
1	Extract *Aesculus* leaf (quercitrin)(6)	42.6[a]	40.6[a]
2	Extract *Aesculus* seed (quercetin) (6)	0[a]	1.3[a]
3	Quercitrin (0.8 mg.) Ascorbic acid (0.25 mg.)/ml.	103.1[a]	99.4[a]
4	Rutin (×10)	100.4	100.4
5	Rutin (50 mg.) Ascorbic acid (25 mg.)/ml.	98.0	100.0
6	Rutin (70 mg.) Ascorbic acid (70 mg.)/tab.	98.9	100.1
7	Rutin (20 mg.) Ascorbic acid (20 mg.) Hesperidin (50 mg.)/tab.	101.8	103.7
8	Carbazochrome 3-sodium sulfonate (25 mg.) Hesperidin (50 mg.) Rutin (40 mg.) Ascorbic acid (100 mg.) Vitamin K (5 mg.) Dibasic calcium phosphate (300 mg.)/Gm.	100.3	103.7
9	Methylhesperidin (40 mg.) Rutin (40 mg.) Ascorbic acid (100 mg.) Adrenochrome monosemicarbazone (2 mg.) Vitamin K_4 diacetate (2 mg.)/Gm.	124.9	116.0
10	Rutin (100 mg.) Scutellaria root (50 mg.) Coptis (25 mg.) Rheum (25 mg.) Extract rheum, coptis, scutellaria root (85 mg.)/Gm.	120.3	120.3
11	Rutin (20 mg.) Scutellaria root (10 mg.) Coptis (5 mg.) Rheum (5 mg.) Extract rheum, coptis, scutellaria root (17 mg.)/tab.	114.4	118.1

[a] Each value represents a % of quercitrin (Nos. 1 and 3) and quercetin (No. 2) in the preparations.

volumetric flask and diluting with water to make 100 ml.

(A) Zirconium Chloride—To 5 ml. of the sample solution in a 25-ml. volumetric flask, 1 ml. of a buffer solution [*a*, 2 *M* acetic acid; *b*, 2 *M* acetic acid–2 *M* sodium acetate (3:1); *c*, 2 *M* acetic acid-2 *M* sodium acetate (1:1); *d*, 2 *M* acetic acid–2 *M* sodium acetate (1:3); *e*, 2 *M* sodium acetate] and 1 ml. of 3% zirconium chloride were added, and diluted with water to the mark.

(B) β-Aminoethyl Diphenylborate—To 5 ml. of the sample solution in a 25-ml. volumetric flask, 1 ml. of a buffer solution (same as above) and 1 ml. of methanolic 1% β-aminoethyl diphenylborate were added, and diluted with methanol to the mark.

CONCLUSION

As shown in Fig. 1, 2 *M* acetic acid–2 *M* sodium acetate (3:1) solution (*b*) was suitable for method *A*. On the other hand, 2 *M* sodium acetate solution (*e*) was suitable for method *B* as shown in Fig. 2. Calibration curves for I are shown in Fig. 3.

It is considered that further investigation should be carried out to introduce a new quantitative analytical method for rutin preparations and/or vegetable crude drugs. In addition to the two methods mentioned above, there is another method for the quantitative analysis of flavonoids under investigation in our laboratories.

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Keyphrases

Flavonoids—analysis
Colorimetric analysis
Zirconium chloride—color reagent
β-Aminoethyl diphenylborate—color reagent
pH profile—colored complex