Colorimetric and Spectrophotometric Determinations of Hydrastis Alkaloids in Pharmaceutical Preparations

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Abstract □ Hydrastine, canadine, and berberine were determined by the acid-dye technique. At pH 5.6, both the tertiary and the quaternary hydrastis alkaloids formed ion-pairs with bromcresol purple. The liberated hydrastine and canadine from the alkaloid–dye complexes were determined spectrophotometrically in the presence of berberine by the three-wavelength method of analysis. However, berberine could not be assayed successfully by this method due to significant partitioning in the aqueous phase during extraction. At pH 7.2, only berberine could ion-pair with bromcresol purple to form a chloroform-extractable complex. Consequently, the berberine content was analyzed selectively through the colorimetric determination of the combined dye at 580 nm. The suitability of the proposed methods was examined through the analyses of synthetic mixtures of hydrastis alkaloids and samples of hydrastis tincture and liquid extract. Percentage recoveries were 98.2–101.4 for the synthetic mixtures and 98.4–101.7 for the tincture and liquid extract spiked with berberine.

Keyphrases □ Colorimetry—analysis, berberine in pharmaceutical preparations and synthetic mixtures of hydrastis alkaloids, effect of pH on alkaloid–dye complex formation □ Spectrophotometry—analyses, hydrastine and canadine in pharmaceutical preparations and synthetic mixtures of hydrastis alkaloids, effect of pH on alkaloid–dye complex formation □ Hydrastis alkaloids—spectrophotometric and colorimetric determinations of alkaloid–dye complexes, effect of pH on complex formation □ Berberine—hydrastis alkaloid, colorimetric determination, effect of pH on alkaloid–dye complex formation □ Canadine—hydrastis alkaloid, spectrophotometric determination, effect of pH on alkaloid–dye complex formation □ Hydrastine—hydrastis alkaloid, spectrophotometric determination of alkaloid–dye complex, effect of pH on complex formation

The active ingredients and therapeutic uses of hydrastis were reviewed previously (1). The drug and its preparations are official in several pharmacopeias (2). Pharmacopeial assay methods for hydrastis rhizome or its liquid extract determine the hydrastine content while neglecting other alkaloids (3, 4).

BACKGROUND

The BPC (3) assays for hydrastine involve either a gravimetric step (for the liquids) or a titrimetric end-point (for powdered hydrastis). As with most gravimetric methods, the residue obtained was found to be impure since more than one spot was revealed by TLC. The titrimetric assay suffers from the disadvantage that the end-point is masked by the color of the extract.

The procedure of the French Pharmacopoeia (4) depends on the selective extraction by ether of hydrastine from the powdered drug after alkalinization with ammonia. Stanislas *et al.* (5) suggested a modification of the method in which the extracted hydrastine was determined spectrophotometrically at 295 and 313 nm. However, this modified method is relatively time consuming; it requires at least 7 hr.

Paper chromatography followed by colorimetric or spectrofluorometric determination was suggested (6, 7) for the assay of hydrastis. Direct spectrofluorometric determination of berberine, hydrastine, and canadine in synthetic mixtures was attempted by Gaille *et al.* (8). However, when applied to the hydrastis extract, only berberine and canadine could be determined by this method.

Column chromatography followed by spectrophotometric determination of hydrastine and berberine in liquid extracts of hydrastis also was suggested (9). Berberine and coptisine in coptis rhizome were separated by constant-potential electrophoresis followed by fluorescence densitometry at 550 nm (10). TLC was used to separate berberine prior to its determination as the tetrabromophenolphthalein ethyl ester ion-pair (11). Sakai *et al.* (12) determined berberine in pharmaceutical preparations as an ion-pair using 2,6-dichloroindophenol sodium. The ion-pair complex was stable for only 30 min.

The objective of the present work was to devise a simple and sensitive method for the determination of hydrastis alkaloids without chromatographic separation. Although the UV spectra of berberine, hydrastine, and canadine suggest that they can be determined spectrophotometrically by applying the three-wavelength method, it was possible to estimate only hydrastine and canadine in preparations of hydrastis. Because berberine is water soluble (1 in 4.5), it could not be determined by this method due to its incomplete recovery during extraction. Alternatively, berberine was selectively assayed colorimetrically using the acid-dye technique since, at pH 7.2, neither hydrastine nor canadine formed chloroform-extractable complexes with bromcresol purple.

EXPERIMENTAL

Materials—McIlvain buffers of pH 5.6 and 7.2 were prepared by mixing the required volumes of 0.1 *M* citric acid and 0.2 *M* dibasic sodium phosphate. Hydrastis liquid extract BPC, hydrastis tincture BPC, and analytical reagent grade sodium chloride were used. Hydrastine[1], canadine[2], and berberine acid sulfate[1] were used as received.

Solution Preparation—A berberine stock solution was prepared by dissolving 0.25 g of berberine acid sulfate in 100 ml of water. A berberine reference solution (1.25 mg %) was prepared by diluting 0.5 ml of the stock solution to 100 ml with 1 *N* HCl.

The canadine and hydrastine stock solutions were prepared by dissolving 0.2 g of either alkaloidal base in 100 ml of 1 *N* HCl. The canadine and hydrastine reference solutions were prepared by diluting 2 ml of the respective stock solution to 100 ml with 1 *N* HCl. Each reference solution contained 4 mg % of the alkaloidal base.

The bromcresol purple solution was prepared by dissolving 50 mg of the dye[3] in a few drops of 0.1 *N* NaOH and bringing the solution to 100 ml with water.

Methods—*Berberine Content of Hydrastis Tincture and Liquid Extract*—A 5-ml aliquot of hydrastis tincture (or 0.5 ml of hydrastis liquid extract diluted to 5 ml with 60% ethanol) was diluted to 1 liter with pH 7.2 buffer. A 10-ml aliquot (containing 0.05–0.2 mg of berberine) was transferred to a 50-ml separator containing 10 ml of chloroform and 1 ml of the dye solution. After shaking for 1 min, the layers were allowed to separate completely (15 min).

The chloroform layer was transferred to a second separator, and the aqueous layer was reextracted with another 10 ml of chloroform. The combined chloroform extracts were shaken for 15 sec with 10 ml of 0.1 *N* NaOH to liberate the combined dye. The aqueous layer was transferred to a 25-ml volumetric flask. After adjustment to volume, the intensity of the color of the solution was measured at 590 nm. The berberine content was determined by assaying a standard berberine solution concomitantly.

Hydrastine and Canadine Content of Hydrastis Tincture and Liquid Extract—Five milliliters of the tincture (or 0.5 ml of the liquid extract diluted to 5 ml with 60% ethanol) was diluted to 500 ml with pH 5.6 buffer. A 10-ml aliquot (containing 0.2–0.6 mg of total hydrastis alkaloids) was transferred to a 50-ml separator containing 1 ml of the dye solution and 10 ml of chloroform. After shaking for 1 min, the layers were allowed to separate completely.

The chloroform layer was transferred to a second separator, and the extraction was repeated with 2 × 10 ml of chloroform. The combined chloroform layers were extracted with 10 ml of 0.1 *N* NaOH for 15 sec,

[1] Merck, Darmstadt, West Germany.
[2] Provided by Dr. Peter W. Jeffs, Chemistry Department, Duke University, Durham, N.C.
[3] British Drug Houses Ltd., Poole, England.

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Table I—Results of Replicate Assays of Hydrastine, Canadine, and Berberine in Hydrastis Tincture

Assay	Alkaloid Concentration, g % (w/v)		
	Hydrastine[a]	Canadine	Berberine
1	0.229	0.054	0.265
2	0.227	0.050	0.261
3	0.218	0.077	0.262
4	0.223	0.052	0.266
5	0.218	0.054	0.265
6	0.223	0.052	0.262
Mean	0.223	0.056	0.263

[a] The hydrastine content found by the BPC method was 0.21 g % (w/v).

followed by 5 ml of saturated sodium chloride solution. The chloroform layer was evaporated on a water bath just to dryness. The residue was dissolved in 1 *N* HCl, transferred to a 25-ml volumetric flask, and brought to volume. The absorbances of the final solution together with the canadine, hydrastine, and berberine reference solutions were measured concomitantly in 1-cm cells at 285, 295, and 345 nm with a suitable spectrophotometer[4] using 1 *N* HCl as the blank.

The concentrations of canadine, C_a, and hydrastine, C_b, expressed in gram percent (w/v), were calculated from:

$$C_a = \frac{\left(A_1 - A_3\frac{\gamma_1}{\gamma_3}\right)\beta_2 - \left(A_2 - A_3\frac{\gamma_2}{\gamma_3}\right)\beta_1}{\alpha_1\beta_2 - \alpha_2\beta_1} \times \frac{1}{4} \times \frac{500}{10} \times \frac{100}{5} \quad \text{(Eq. 1)}$$

$$C_b = \frac{\left(A_2 - A_3\frac{\gamma_2}{\gamma_3}\right)\alpha_1 - \left(A_1 - A_3\frac{\gamma_1}{\gamma_3}\right)\alpha_2}{\alpha_1\beta_2 - \alpha_2\beta_1} \times \frac{1}{4} \times \frac{500}{10} \times \frac{100}{5} \quad \text{(Eq. 2)}$$

where:

$$A_1 = C_a\alpha_1 + C_b\beta_1 + C_c\gamma_1$$
$$A_2 = C_a\alpha_2 + C_b\beta_2 + C_c\gamma_2$$
$$A_3 = C_c\gamma_3$$

and where A_1, A_2, and A_3 are the absorbances of the final solution at 285, 295, and 345 nm, respectively, and α, β, and γ are the absorptivities of canadine, hydrastine, and berberine, respectively. The subscripts 1, 2, and 3 refer to the respective wavelengths at 285, 295, and 345 nm.

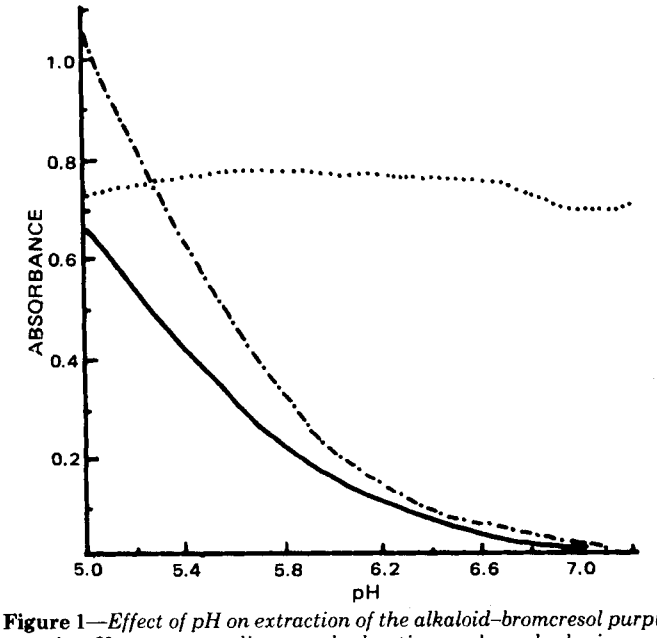

Figure 1—*Effect of pH on extraction of the alkaloid–bromcresol purple complex. Key: —, canadine; -·-, hydrastine; and ·····, berberine.*

[4] Beckman model 24 spectrophotometer.

RESULTS AND DISCUSSION

The λ_{max} values were 230 and 345 (berberine), 285 (canadine), and 295 (hydrastine) nm. Analysis of a synthetic mixture containing 0.2% (w/v) hydrastine, 0.05% (w/v) canadine, and 0.25% (w/v) berberine by the proposed method gave percentage recoveries of 98.2 and 101.4 for canadine and hydrastine, respectively. Direct UV estimation was successful for berberine in synthetic mixtures. However, in preparations where an extraction step was involved, berberine could not be determined successfully (<80% recovery). This result was due mainly to its partitioning between the aqueous and organic phases.

Table I shows the results of replicate assays of hydrastis tincture for hydrastine and canadine by the suggested method. The mean gram percent of hydrastine found (±*SD*) was 0.223 (±0.004). This result compares favorably with the result obtained by the BPC method (0.21% w/v). The slightly lower percentage recovery found by the BPC method might be attributed to incomplete extraction of hydrastine during the assay. For canadine, which oftens represents one-third to one-fourth of the hydrastine content, the results showed more scattering (Table I).

Figure 1 shows the effect of pH on the extractability of alkaloid–bromcresol purple complexes for berberine, canadine, and hydrastine. The formation of the complexes was strongly pH dependent for canadine and hydrastine, with an optimum pH range of 5–5.6. At pH values above 7, any complexes formed between canadine and hydrastine and the dye were not recovered in the chloroform phase. For berberine, the alkaloid–dye complex apparently was formed within the whole pH range studied (5.0–7.2), with the complex extractability being only slightly pH dependent.

The selective formation of the berberine–bromcresol purple complex at pH 7.2 was applied to the assay of this alkaloid in the presence of canadine and hydrastine. Replicate assays for berberine in a synthetic mixture containing the three alkaloids gave a mean percentage recovery (±*SD*) of 100.7 (± 0.31) for six determinations. Results of the berberine assay in the hydrastis tincture are shown in Table I. Recovery experiments made for hydrastis tincture spiked with various berberine concentrations gave a percentage recovery of 98.4–101.7.

The proposed procedures have the advantage of being less time consuming, with the assay requiring an average of 1 hr. The BPC procedure requires >5 hr and 100 ml of the tincture whereas 0.1 ml is required in the proposed method. This paper also presents a sensitive method for the assay of berberine in the presence of other tertiary hydrastis alkaloids.

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