A RAPID SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATIVE ESTIMATION OF OCTADECYL p-COUMARATES

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A UV spectrum of octadecyl p-coumarates gives a peak at $\lambda_{max}(MeOH) = 308.6$ nm (log $\varepsilon = 4.13$). On addition of alkali, the peak undergoes a bathochromic shift to 358.2 nm with a hyperchromic effect. A linear relationship found between the concentration of octadecyl p-coumarates and the hyperchromic effect can be used to quantitatively estimate octadecyl p-coumarates in samples devoid of other compounds exhibiting the similar hyperchromic effect. The method may also be useful for quantitative comparison of total alkyl coumarates in plant samples.

Keywords: octadecyl p-coumarates, rapid spectrophotometric method, quantitative estimation, hyperchromic effect.

БЫСТРЫЙ СПЕКТРОФОТОМЕТРИЧЕСКИЙ МЕТОД КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ОКТАДЕЦИЛ-p-КУМАРАТОВ

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В УФ спектре октадецил-p-кумаратов максимум интенсивности расположен при $\lambda_{max}(MeOH) = 308.6$ нм (log $\varepsilon = 4.13$). При добавлении щелочи происходит батохромный сдвиг максимума к $\lambda = 358.2$ нм, сопровождающийся гиперхромным эффектом. Найденная линейная зависимость между концентрацией октадецил-p-кумаратов и величиной гиперхромного эффекта может быть использована для количественной оценки концентрации октадецил-p-кумаратов в образцах, не содержащих соединений с подобным гиперхромным эффектом. Метод может также применяться для количественного сравнения общего содержания алкилкумаратов в растительных образцах.

Ключевые слова: октадецил-p-кумарат, быстрый спектрофотометрический метод, количественная оценка, гиперхромный эффект.

Introduction. Alkyl coumarates exhibit a wide range of biological activities. Antibacterial [1], insect-resistant [2], phytotoxic [3], melanin formation inhibiting [4], and antioxidant [5] activities have been attributed to alkyl coumarates. Among them, octadecyl p-coumarates
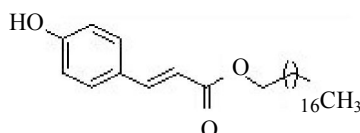
have been reported to be extracted from several plants [6–12] including *Ipomoea carnea* subsp. *fistulosa* [12]. Confirmed biological activities of octadecyl p-coumarates include antifungal [12], DNA polymerase inhibition [13], and DNA topoisomerase and human cancer cell growth inhibition [14] properties.

Quantitative estimations of alkyl coumarates are usually carried out by HPLC [2, 15] or GLC [10]. The main disadvantages of these methods are high costs of instruments and high grade solvents/gases and expertise required for operating the instrument. A recent observation on the *E-Z* isomerization [3, 12, 16] and a substantial difference

in the retention times [12, 16] of the isomers further complicate the estimations by the HPLC method. In this paper, a rapid and simple spectrophotometric method to quantitatively estimate octadecyl p-coumarates is given.

Experimental section. *Isolation and UV spectrophotometry of octadecyl p-coumarates.* Details regarding the isolation of compounds from *Ipomoea carnea* subsp. *fistulosa* and their HPLC purification have been described in the earlier paper [12]. The UV spectrum of octadecyl p-coumarates was taken using a Systronics UV-Visible spectrophotometer.

Determination of hyperchromic effect (ΔA). An aliquot of octadecyl p-coumarates in methanol with the concentration of 50 mg/l was prepared. Solutions of the compound with the required concentrations were obtained by appropriate dilutions. The absorbance at 358.2 nm was noted as A_1. Exactly 4.0 ml of this was taken and exactly 1.0 ml of 0.1N sodium hydroxide was added. This was shaken and the absorbance was noted as A_2 within 5 min. A hyperchromic effect ($A_2 - A_1$) was noted as ΔA.

Estimation in plant samples by spectrophotometric method. Exactly weighed 100 mg of a dried powdered plant material was extracted with methanol (10 extractions) using a Soxhlet apparatus and made up to 100 ml with methanol. ΔA was calculated by the procedure which has already been described.

Estimation by HPLC method. After injecting 50 μl of each of the samples with different concentrations in dichloromethane, peak areas were noted. A linear relationship was found between the concentration and the peak areas. The dried powdered plant material (100 mg) was extracted with dichloromethane (10 extractions) using the Soxhlet apparatus. The extract was concentrated, filtered, and made up to 100 ml with dichloromethane. HPLC analysis was done. The concentrations of octadecyl p-coumarates were calculated from the linear relationship between the concentration and the peak areas.

Spectrophotometric analysis of samples fortified by octadecyl p-coumarates. Exactly weighed 100 mg of the dried powdered plant material were taken and mixed with 0.2 mg of pure octadecyl p-coumarates and Soxhlet extracted with methanol. The extract was concentrated, filtered, and made up to 100 ml with methanol. ΔA was calculated.

Results and discussion. *Hyperchromic effect of alkali on the UV spectrum of octadecyl p-coumarates.* The UV spectrum of octadecyl p-coumarates at the concentration of 10 mg/l given in Fig. 1 shows λ_{max}(MeOH) = 308.6 nm (log ε = 4.13). However in [8], the value of λ_{max}(MeOH) = 311.5 nm (log ε = 4.13) is given. It was observed that at higher concentrations, the vertex of the peak was broad enough to consider both values as correct. On addition of alkali, the peak at 308.6 nm undergoes a bathochromic shift to 358.2 nm with a hyperchromic effect (Fig. 1). The formation of the phenoxide anion on addition of alkali is as shown below:
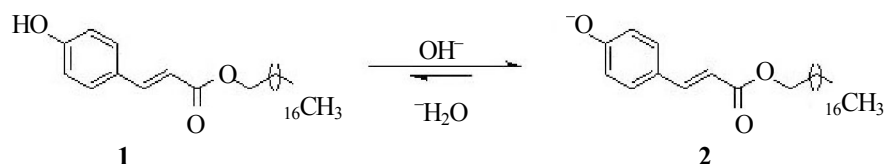
Under alkaline conditions, acidic phenolic hydrogen in **1** is deprotonated to furnish the phenoxide anion **2**. Extended conjugation in **2** increases the energy of the π energy level and hence reduces the energy for a π → π* transition. This reduced energy requirement of the transition manifests itself as the bathochromic shift in the UV spectrum.

Standard curve. Table 1 gives the absorbance of octadecyl p-coumarates at different concentrations ranging from 1.0 to 20 mg/l at 358.2 nm, before and after addition of alkali, and the corresponding differences in the absorption (hyperchromic effect).

TABLE 1. Hyperchromic Effect of Octadecyl p-Coumarates at 358.2 nm on Addition of Alkali

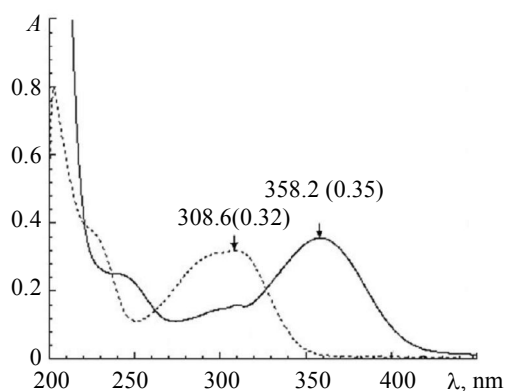| C, mg/l	Absorbance at 358.2 nm before addition of alkali (A_1)	Absorbance at 358.2 nm after addition of alkali (A_2)	Hyperchromic effect ΔA
0	0	0	0
1.0	0.004	0.043	0.039
2.5	0.005	0.097	0.092
5.0	0.007	0.177	0.170
7.5	0.008	0.268	0.260
10.0	0.008	0.350	0.342
12.5	0.009	0.430	0.421
15.0	0.012	0.510	0.498
20.0	0.020	0.663	0.643

Fig 1. Bathochromic shift of the peak in the UV spectrum of octadecyl *p*-coumarates on addition of alkali.
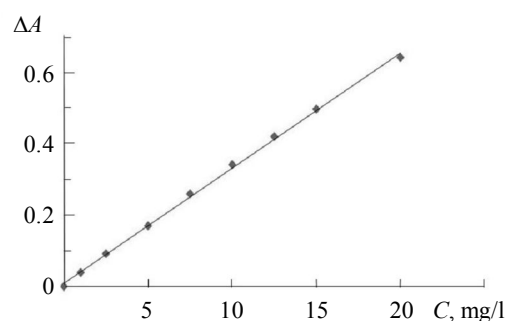

Fig 2. Linear relationship between concentration of octadecyl *p*-coumarates and hyperchromic effect.

Figure 2 shows the linear relationship between and concentration of octadecyl *p*-coumarates and the hyperchromic effect. This is mathematically depicted by the following equation:

$$\Delta A = 0.0098 + 0.0323C, \tag{1}$$

$$R = +0.999,$$

where ΔA is the hyperchromic effect, C is the concentration in mg/l, and R is the correlation coefficient.

Although the hyperchromic effect in the UV spectrum of *p*-coumaric acid has already been reported [17], this may be the first report in which a quantitative relationship between the concentration of coumarates and the hyperchromic effect on addition of alkali has been established.

Estimation in plant sample and comparison with HPLC method. Estimations were carried out both by the HPLC and spectrophotometric methods. The average values obtained by two replications are given in Table 2. It clearly shows that the value obtained by the spectrophotometric method is much higher than that found by HPLC. This is most probably due to other alkyl coumarates present in the plant. It may be noted that other alkyl coumarates namely methyl, octyl, and dodecyl coumarates have also been reported recently in the same plant [18].

TABLE 2. Comparison of Estimations by Spectrophotometry and HPLC

Method	Percentage
Spectrophotometry	0.106 ± 0.004
HPLC	0.046 ± 0.002

Thus the spectrophotometric method cannot be used to estimate octadecyl *p*-coumarates in plant samples which contain other compounds with similar hyperchromic effect at 358.2 nm on addition of alkali. Thus the value of $0.106 \pm 0.004\%$ indicates only total alkyl coumarates in the plant expressed as octadecyl *p*-coumarates.

Spectrophotometric estimation of samples fortified by octadecyl p-coumarates. Table 3 gives a comparison of the estimations for both the plant sample taken alone and that fortified with octadecyl *p*-coumarates. Out of the 0.292 mg estimated for the fortified sample (0.292%), 0.106 mg came from the plant material and the remaining 0.186 mg came from the 0.2 mg of octadecyl *p*-coumarates added to the sample. This shows that 93% of octadecyl *p*-coumarates used for fortification were recovered and estimated by the spectrophotometric method.

TABLE 3. Comparison of Estimations in Plant Material and in Fortified Sample by Spectrophotometric Method

Materials taken for estimation	ΔA	Percentage obtained
100 mg of plant material	0.044 ± 0.001	0.106 ± 0.004
100 mg of plant material + 0.2 mg of octadecyl *p*-coumarates	0.104 ± 0.001	0.292 ± 0.004
Percent recovery of octadecyl *p*-coumarates used for fortification	–	93.0%

Proposed procedure for estimation of octadecyl p-coumarates in samples devoid of other compounds with hyperchromic effect at 358.2 nm. Dry the sample and powder it. Take 100.0 mg of the material and extract with methanol (10 extractions) using the Soxhlet apparatus. Concentrate and filter it and make up to 100 ml with methanol. Note the absorbance at 358.2 nm as A_1. Take exactly 4.0 ml of the solution and add 1.0 ml of 0.1 N sodium hydroxide. Note the absorbance at 358.2 nm as A_2. Note the difference in the absorbances as ΔA. Calculate the concentration of octadecyl *p*-coumarates from the equation (1).

Proposed procedure for estimation of total alkyl coumarates in plant samples. Dry the sample and powder it. Take 100.0 mg of the material and extract with methanol (10 extractions) using the Soxhlet apparatus. Concentrate and filter it and make up to 100 ml with methanol. Find out ΔA by the procedure given above. Applying this value to the Eq. (1) will give the total alkyl coumarates in the sample expressed as octadecyl *p*-coumarates.

Conclusion. The rapid spectrophotometric method takes advantage of the linear relationship between the concentration of octadecyl *p*-coumarates and the hyperchromic effect at 358.2 nm on addition of alkali and can be used to quantitatively estimate octadecyl *p*-coumarates. The method cannot be used for the estimation of octadecyl *p*-coumarates in plant samples where other coumarates or compounds showing similar hyperchromic effect at 358.2 nm are present. However, the method may be useful in quantitative comparison of total coumarates in plant samples.

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