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Determination of Aspirin and Salicylic Acid in Aspirin Tablets by Second Derivative Ultraviolet Spectrometry

KEISUKE KITAMURA,* MOTOKO TAKAGI and KEIICHIRO HOZUMI

Kyoto College of Pharmacy, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607, Japan

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The second derivative ultraviolet (UV) spectra of aspirin and salicylic acid in a solution of powdered aspirin tablets were not interfered with by the intense and time-dependent signal backgrounds caused by excipients and antacids contained in the tablets. The promotion of aspirin hydrolysis to salicylic acid in the solution by antacids could be sufficiently suppressed by using ethanol containing 1% (w/v) of citric acid as a solvent. Three kinds of commercial aspirin tablets were assayed by second derivative UV spectrometry without any procedure for separation of the pharmaceuticals from the tablet additives. The contents of aspirin and salicylic acid could be determined with relative standard deviations of $<0.5\%$ for aspirin and $<3.0\%$ for salicylic acid.

Keywords——derivative spectrometry; second derivative spectrum; aspirin; aspirin tablet; salicylic acid

The determination of aspirin and its major decomposition product, salicylic acid, in aspirin tablets has been investigated by gas-liquid chromatography (GLC),[1,2] liquid chromatography (LC),[3] and high-performance liquid chromatography (HPLC).[4] In these chromatographic methods, separation of the pharmaceuticals from tablet additives (excipients and antacids) is essential. Since GLC methods require chemical derivatizations of aspirin and salicylic acid, the separation is needed in order to avoid interference of the additives with the chemical derivatizations. In the LC and HPLC methods, insoluble additives should be completely removed to prevent the columns from becoming blocked. The extraction procedures must be carried out with great care so as not to induce hydrolysis of aspirin to salicylic acid during the solvent extractions, especially when the tablets contain buffers or antacids.[2] Furthermore, in the LC and HPLC methods, mobile phases employing methanol and water, even in small quantities, may cause hydrolysis of aspirin.[4]

Recently we have reported that the very small amount of salicylic acid (less than 0.1% of aspirin content) in pharmaceutical aspirin powder could be determined by second derivative ultraviolet (UV) spectrometry, even though the signal of salicylic acid in the absorption spectrum severely overlaps with the large aspirin signal.[5] Since derivative techniques in spectrophotometry are useful not only for overlapping signal but also for signals with high backgrounds,[6] we further extended the derivative spectrometry to the analysis of aspirin and salicylic acid in aspirin tablets, where the insoluble fine powder of the tablet additives would cause an intense and time-dependent signal backgrounds.

Derivative spectrometry provides a simple and time-saving assay method for aspirin and salicylic acid in aspirin tablets, and does not require troublesome extraction procedures or time-consuming chromatographic conditionings.

Experimental

Chemicals and Solvent——Aspirin was twice recrystallized from acetone and salicylic acid was twice recrystallized from cyclohexane. Aluminum aminoacetate was synthesized according to the literature.[7] Ethanol containing

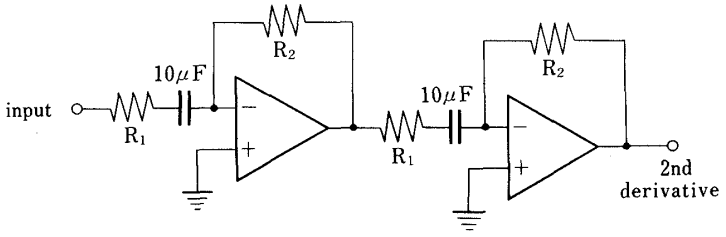

Fig. 1. Simplified Derivative Circuit

Three kinds of circuit having various resistance value ($R_1=37\,k\Omega$, $R_2=270\,k\Omega$; $R_1=138\,k\Omega$, $R_2=820\,k\Omega$; $R_1=400\,k\Omega$, $R_2=2200\,k\Omega$) are installed and can be selected by switching.

1% (w/v) citric acid was used as the solvent for measurements of spectra.

Preparation of Standard Solutions——Aspirin standard solutions were prepared just before measurement. A suitable amount of aspirin was weighed accurately, transferred into a 50- or 250-ml volumetric flask, dissolved and diluted to volume with the solvent. Salicylic acid standard solutions were prepared by appropriate dilutions of the stock solution with the solvent.

Preparation of Assay Solutions——The average weight of five tablets was determined, and the tablets were ground to a fine powder by a mortar and pestle. A portion of the powder equivalent to about 300 mg of aspirin was weighed accurately and transferred into a 50-ml volumetric flask. The solvent was added to 50 ml and the mixture was shaken vigorously for about 30 s. Then a 5.0-ml portion of the solution was diluted to 25.0 ml (tablets not containing antacid) or 50.0 ml (tablets containing antacid) with the solvent for the assay of salicylic acid. For the assay of aspirin, a 5.0-ml portion of the solution just prepared for the salicylic acid assay was further diluted to 100.0 ml (for the solution which had been diluted to 25.0 ml) or 50.0 ml (for the solution which had been diluted to 50.0 ml). The spectrum was measured immediately after preparation of the sample solution.

Second Derivative UV Spectrometry——An electronic differentiator[5] was connected to a UV-VIS double-beam scanning spectrophotometer (Shimadzu UV-210A). A simplified diagram of the derivative circuit is shown in Fig. 1. Second derivative spectra were obtained at a slit width of 1 nm, a scanning speed of 120 nm/min, and a time constant of 22.0 s ($R_2=2200\,k\Omega$).

Results and Discussion

The Absorption (Zero Order) and Second Derivative Spectra of Aspirin and Salicylic Acid with and without Tablet Additives

Aspirin tablets are usually manufactured from aspirin powder and tablet excipients (about 80 : 20 content ratio), and frequently contain antacids (buffered aspirin tablets). These insoluble tablet additives cause high signal backgrounds in zero order spectra of aspirin and salicylic acid, as can be seen in the lower parts of Fig. 2a and b, respectively. The intensities of the backgrounds were time-dependent because the coarse particles of the additives gradually settled to the bottom of the cuvette. They will also vary from sample to sample according to the kinds and amounts of additives in tablets. However, when the second derivative spectra of these samples were measured, the effect of the backgrounds seemed to disappear, as can be seen in the upper parts of Fig. 2a and b. Then, the elimination of background effects by use of the second derivative spectra was quantitatively investigated using three kinds of additives which are commonly used in commercial aspirin tablets.

Influence of Tablet Additives on the Second Derivative Signal Amplitudes of Aspirin and Salicylic Acid

The excipients usually used in aspirin tablets are (1—2%), magnesium stearate (0.5—1%), crystalline cellulose (−10%) and corn starch (−10%). For experimental convenience, a mixture of these excipients was prepared in a ratio of 2 : 1 : 10 : 10 in the same order. The antacids generally incorporated in buffered aspirin tablets are dried aluminum hydroxide gel, magnesium carbonate and aluminum aminoacetate. According to those generally found in commercial aspirin tablets, three kinds of additives were prepared from the above materials,

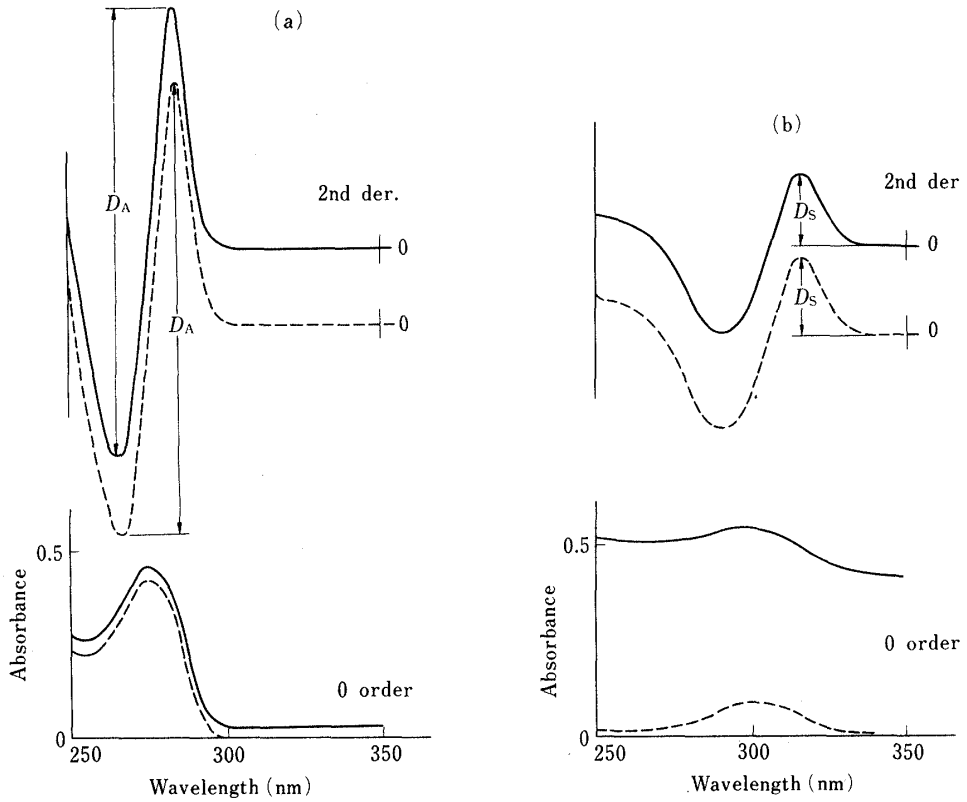

Fig. 2. Zero Order and Second Derivative UV Spectra of Aspirin and Salicylic Acid in the Presence and Absence of Tablet Additives

(a) ---- aspirin (66.5 μg/ml) alone, —— aspirin (66.5 μg/ml)+excipients mixture (19.9 μg/ml)+magnesium carbonate (21.7 μg/ml)+aluminum aminoacetate (10.2 μg/ml).
(b) ---- salicylic acid (3.0 μg/ml) alone, —— salicylic acid (3.0 μg/ml)+excipients mixture (188 μg/ml)+magnesium carbonate (212 μg/ml)+aluminum aminoacetate (96 μg/ml).

TABLE I. Effect of Tablet Additives on the Second Derivative Signal Amplitude of Aspirin (D_A)

Concentration of aspirin (μg/ml)	Amounts of additives added (μg/ml)				D_A[a] mm
	Excipients mixture	Dried aluminum hydroxide gel	Magnesium carbonate	Aluminum aminoacetate	
59.92	—	—	—	—	133.2±0.4
	23.9	—	—	—	131.8±0.3
60.42	—	—	—	—	134.1±0.3
	20.7	9.8	10.2	—	133.6±0.1
60.36	—	—	—	—	135.6±0.4
	24.4	—	19.8	10.6	134.0±0.2

a) Mean value with confidence limits at the 95% level ($n=5$).

i.e., excipients mixture alone, excipients mixture + dried aluminum hydroxide gel + magnesium carbonate (2 : 1 : 1), and excipients mixture + magnesium carbonate + aluminum aminoacetate (2 : 2 : 1).

To examine the effects of these additives on the aspirin signal, the second derivative spectra of standard aspirin solutions were measured with and without the incorporation of

TABLE II. Effect of Tablet Additives on the Second Derivative Satellite Peak Height of Salicylic Acid (D_S)

Concentration of salicylic acid (μg/ml)	Amount of additives added (μg/ml)				D_S[a] mm
	Excipients mixture	Dried aluminum hydroxide gel	Magnesium carbonate	Aluminum aminoacetate	
2.00	—	—	—	—	16.4±0.3
	198	—	—	—	16.4±0.4
	200	101	84	—	16.0±0.3
	218	—	191	111	16.5±0.4
3.00	—	—	—	—	24.8±0.4
	216	—	—	—	24.6±0.5
	214	103	95	—	24.6±0.5
	229	—	195	126	24.4±0.4
5.00	—	—	—	—	40.5±0.3
	198	—	—	—	40.6±0.5
	195	97	113	—	40.7±0.8
	229	—	199	93	40.4±0.4

a) Mean value with confidence limits at the 95% level ($n=5$).

TABLE III. Suppressing Effect of Citric Acid on Aspirin Hydrolysis Induced by Tablet Additives

Concentration of aspirin (μg/ml)	Amounts of additives added (μg/ml)				ΔD_S[a] mm
	Excipients mixture	Dried aluminum hydroxide gel	Magnesium carbonate	Aluminum aminoacetate	
1201.2	—	—	—	—	1.1
	792	—	—	—	0.9
	812	428	400	—	0.7
	788	—	792	408	1.9

a) Increase in D_S value for 90 min.

these additives, and the signal amplitudes of aspirin (denoted by D_A in Fig. 2a) were compared. The spectrum was measured five times for each sample; the time required for the five measurements was about 15 min. The results are summarized in Table I. The D_A value did not show any appreciable difference between the samples with and without the additives, so that it was confirmed that the background effects on the aspirin signal were efficiently eliminated by the differentiation.

The effect of backgrounds due to these additives on the salicylic acid signal was checked by measuring the height of the satellite peak at 316 nm (denoted by D_S in Fig. 2b), not the peak-to-trough amplitude as in the case of aspirin, because the trough of the second derivative salicylic acid signal was interfered with by the large signal of coexisting aspirin.[5] The results in Table II clearly show that the D_S value was not affected by the background contributions of these additives.

Suppression of Aspirin Hydrolysis Induced by Tablet Additives

The hydrolysis of aspirin to salicylic acid was sufficiently suppressed in 1% (w/v) chloroacetic acid–ethanol solution.[5] However, the incorporation of antacids, magnesium

carbonate and aluminum aminoacetate, induced marked aspirin hydrolysis in ethanol, dioxane or chloroform solutions containing more than 1% (w/v) chloroacetic acid. The problem was solved by using citric acid instead of chloroacetic acid, as confirmed by the following experiment. Aspirin was dissolved in 1% (w/v) citric acid in ethanol solution with and without the tablet additives, and the increase of salicylic acid concentration was monitored in terms of D_S value. The results in Table III show that the increase in D_S value during 90 min was within 2 mm, which is lower than the previous results for aspirin powder alone in 1% chloroacetic acid–ethanol solution (2 mm increase in 60 min).[5] Thus, it was apparent that 1% citric acid–ethanol solution could effectively suppress aspirin hydrolysis in the presence of the tablet additives.

Calibration Curves for Aspirin and Salicylic Acid

To obtain a calibration curve for aspirin, the second derivative spectra of standard aspirin solutions were taken at five concentrations (20.0—100.0 μg/ml). The spectrum was measured five times at each concentrations. The correlation coefficient between the D_A value and aspirin concentration was 0.9999 and a good straight line which passed through the origin was obtained. The slope was 2.24 mm/(μg/ml) with a relative standard deviation ($s/\bar{x}$) of 0.7%. These values correspond to confidence limits of 2.24+0.02 mm/(μg/ml) at the 95% level. Thus, the aspirin concentraton C_A (μg/ml) can be calculated from 1/slope $\times D_A$, that is

$$C_A = 0.447 \times D_A \quad (1)$$

Since the calibration curve for salicylic acid in 1% chloroacetic–ethanol solution was already reported,[5] the calibration curve was checked in 1% citric acid–ethanol solution. No difference between the results in the two solvents was found. Thus, salicylic acid concentration C_S (μg/ml) could be obtained from the relation, $C_S = 0.125 \times D_S$.[5]

Effect of Salicylic Acid on D_A Value

There was a possibility that D_A value might be influenced by coexisting salicylic acid, since the trough of the second derivative signal of salicylic acid appeared at the same position as the aspirin signal, as can be seen in Fig. 2. The effect on D_A value was investigated by the incorporation of salicylic acid into aspirin standard solutions up to about 8% of aspirin

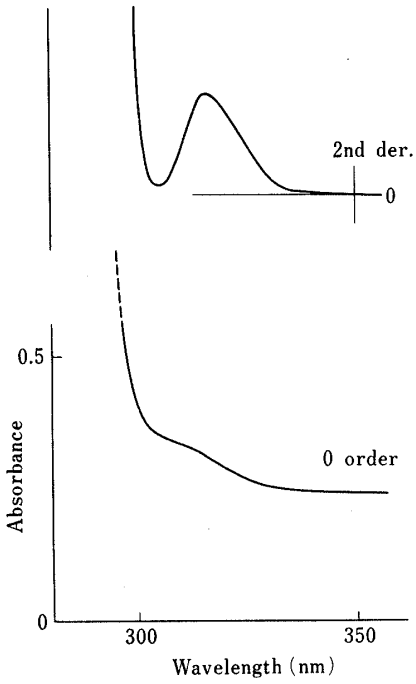

Fig. 3. Zero Order and Second Derivative UV Spectra of Salicylic Acid in a Commercial Aspirin Tablet

TABLE IV. Assay Results for Commercial Aspirin Tablets

Tablet[a]	Claimed content of aspirin per tablet (mg)	Amount of sample (mg)	Aspirin found per tablet % of Claim	Salicylic acid found per tablet % of aspirin
A	500	374.4	103.6±0.3[b]	0.054±0.003[b]
		381.0	102.9±0.5	0.051±0.003
		382.3	102.7±0.4	0.053±0.004
			m=103.1[c]	m=0.053[c]
			r.s.d.=0.5%[d]	r.s.d.=2.9%[d]
B	324	502.0	98.7±0.3[b]	0.691±0.007[b]
		500.5	98.8±0.3	0.695±0.015
		507.6	99.3±0.3	0.706±0.020
			m=98.9[c]	m=0.697[c]
			r.s.d.=0.3%[d]	r.s.d.=1.1%[d]
C	330	480.6	96.5±0.1[b]	0.251±0.006[b]
		482.7	96.7±0.2	0.236±0.008
		491.0	96.7±0.2	0.244±0.009
			m=96.6[c]	m=0.244[c]
			r.s.d.=0.1%[d]	r.s.d.=3.0%[d]

a) Additives claimed: A (126 mg of excipients alone), B (35 mg of dried aluminum hydroxide gel, 35 mg of magnesium carbonate and 161 mg of excipients), C (50 mg of aluminum aminoacetate, 100 mg of magnesium carbonate and 58 mg of excipients).
b) Mean value with confidence limits at the 95% level ($n=5$).
c) Mean value for three determinations.
d) Relative standard deviation for three determinations.

content. The results showed that the D_A value decreased linearly with respect to salicylic acid concentration, and the correction term for C_A of a sample solution containing salicylic acid of C_S' (μg/ml) was found to be $+1.6 \times C_S'$ (μg/ml). This implies that the error in C_A value induced by neglecting the salicylic acid effect would be 1% or so at most, because salicylic acid content in aspirin tablets is usually below 1%. Nevertheless, the correction is preferable for accurate results.

Assay of Commercial Aspirin Tablets

Three kinds of commercially obtained aspirin tablets, A, B and C, were assayed. A typical spectrum for the assay of salicylic acid is shown in Fig. 3. The results are summarized in Table IV. The correction of aspirin concentration for salicylic acid concentration was made in every case. The statistical analysis of assay results show satisfactory precisions of the second derivative method for the determinations of aspirin and salicylic acid in aspirin tablets. The aspirin contents were also measured by the GLC method[2] for comparison and obtained as 102.2% for tablet A, 97.2% for B, and 95.1% for C. The values correspond well to those obtained by the second derivative UV spectrometry.

Consequently, it was demonstrated that the assay of aspirin and salicylic acid in aspirin tablets could be simply performed by second derivative UV spectrometry without the need for troublesome separation of the pharmaceuticals from tablet additives.

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