Spectrophotometric Determination of *p*-Aminophenol Alone or in the Presence of Acetaminophen

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Abstract □ Small amounts of *p*-aminophenol were analyzed quantitatively by mixing with 4-nitro- or 2,4-dinitrobenzaldehyde in neutral alcoholic solution and then quantitatively forming the respective Schiff bases, 4′-nitro- and 2′,4′-dinitrobenzylidene-4-hydroxyaniline, by evaporating the alcoholic solutions to dryness. The Schiff bases thus formed were examined spectrophotometrically. This procedure was also used for the determination of *p*-aminophenol in the presence of its degradation products. It was necessary, however, to separate the respective Schiff bases by TLC from the degradation products and the reaction mixture prior to spectrophotometric examination. Small amounts of *p*-aminophenol present in acetaminophen were quantitatively determined by applying the procedure to a water–ethanol (95:5 v/v) extract of the samples of acetaminophen. TLC was used to separate the respective Schiff bases from the reaction mixture and acetaminophen prior to spectrophotometric examination.

Keyphrases □ *p*-Aminophenol—spectrophotometric analysis of Schiff base derivatives, alone or in presence of degradation products or acetaminophen, TLC separation □ Spectrophotometry—analysis, Schiff base derivatives of *p*-aminophenol, alone or in presence of degradation products or acetaminophen □ TLC—separation, Schiff base derivatives of *p*-aminophenol in presence of degradation products or acetaminophen

It was reported that *p*-aminophenol (I) liberated in mixtures containing acetaminophen (II) could be determined spectrophotometrically by using 4-nitrobenzaldehyde (1). A color test, with alkaline sodium nitroprusside solution, for small amounts of I in II was described in the British Pharmacopoeia (2), and another test with *p*-dimethylaminocinnamaldehyde was described in NF XIII (3).

Several methods for the determination of I are based on the formation of a colored reaction mixture (3–10), but the exact nature of the reaction products has not been established. A color reaction has also been reported (11). Other methods are based on titrating with perchloric acid (12) or carrying out a polarographic examination (13, 14), but these methods may prove difficult with samples containing small amounts of I.

This paper presents a method for the determination of I either alone or in the presence of its colored degradation products and/or II. This method is based on the reaction between I and 4-nitrobenzaldehyde (III) or 2,4-dinitrobenzaldehyde (IV) with the formation of the colored 4′-nitrobenzylidene-4-hydroxyaniline (V) or 2′,4′-dinitrobenzylidene-4-hydroxyaniline (VI), respectively (Schiff bases).

EXPERIMENTAL[1]

Materials—4-Nitro- and 2,4-dinitrobenzaldehydes[2], acetaminophen[3], petroleum ether[4] (bp 40–70°), and acetic acid[4] were used without further purification. *p*-Aminophenol[5] was purified by three sublimations at 110°/0.1 mm Hg (white crystals, mp 188°). Ethanol[6] and acetone[6] were purified by fractional distillation.

TLC—Separations by TLC were carried out on silica gel[7] layers of 0.50 mm or, when I was present in very small amounts, on silica gel[7] layers of 0.25 mm. The solvent system found suitable for separating V and VI from other interfering components was petroleum ether–acetone (70:30). TLC plates were usually eluted only once but, when necessary, were eluted twice for better separation.

TLC chromatograms were examined against the green fluorescence of the plate (Fig. 1) by viewing in short wavelength[8] (254 nm) UV light and clearly marking the boundaries of the areas containing the different components. It was thus easier to scrape off and then extract the component present in the silica gel in a particular area and determine its UV–visible spectrum. The fluorescence material had no effect on the analytical results.

Preparation of Reagent Solutions—Stock solutions of III and IV were freshly prepared by dissolving 15.1 and 20 mg of material, respectively, in 50 ml of ethanol (final solutions of 2×10^{-3} *M*). These solutions are not stable and must be freshly prepared, preferably, each day.

Standard Solution of I—A stock solution (2×10^{-3} *M*) was prepared by dissolving 10.9 mg of I in 50 ml of ethanol. This solution darkened on standing, but it could be used for 2–3 days. However, it was preferable to prepare it each day.

4′-Nitrobenzylidene-4-hydroxyaniline (V)—A mixture of 0.55 g of I and 0.80 g of III dissolved in 50 ml of ethanol was left standing for 0.5 hr. The alcohol was then evaporated under reduced pressure, and the solid mixture was dissolved in a small amount of ethanol which was again evaporated. The crude solid product obtained (mp 167–168°) was recrystallized from benzene (charcoal) three times, yielding 0.55 g (~60%) (dried at 70°/0.1 mm Hg for ~3 hr), mp 172°.

Anal.—Calc. for $C_{13}H_{10}N_2O_3$: C, 64.46; H, 4.16; N, 11.56. Found: C, 64.74; H, 4.00; N, 11.70.

2′,4′-Dinitrobenzylidene-4-hydroxyaniline (VI)—A mixture of 0.55 g of I and 0.99 g of IV was treated as described for V. The

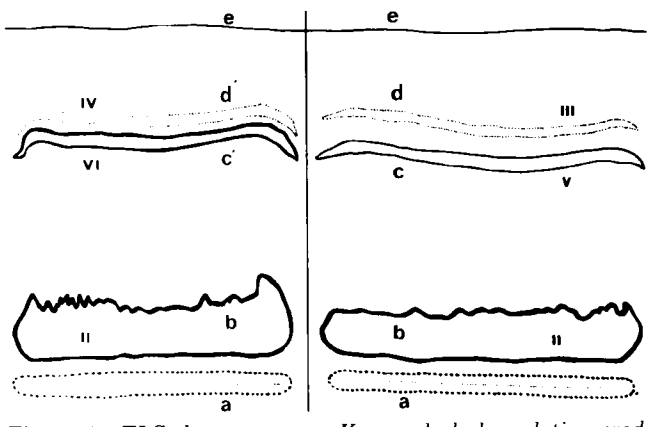


Figure 1—*TLC chromatograms. Key: a, dark degradation products remaining at starting point; and e, solvent front.*

[1] Spectra were determined using a Pye-Unicam SP 8000 recording spectrophotometer. Evaporations were carried out under reduced pressure, using a Büchi Rotavapor R/A, at temperatures not exceeding 40°.

[2] Fluka, puriss.

[3] Fluka, purum.

[4] Carlo Erba.

[5] Fluka, practical.

[6] Technical grade.

[7] GF_{254} Merck reagent.

[8] Chromato-Vue, Ultra-Violet Products, San Gabriel, Calif.

Table I—Deterioration of an Aqueous Solution of I (~8 × 10^{-4} *M*) as Determined by Method 2[a]

Days	Compound V Absorbance[b] at 380 nm	% Loss	Compound VI Absorbance[b] at 404 nm	% Loss
0	0.530	—	0.492	—
3	0.376	29.0	0.370	26.0
5	0.290	45.0	0.266	45.9
7	—	—	0.186	62.2
8	0.148	72.1	—	—

[a] Absorbances were read from full UV–visible spectra. [b] One-centimeter cells as described under Method 2.

yield after the first crystallization was 1 g (~70%). The product was further purified by three recrystallizations (dried at 70°/0.1 mm Hg for ~3 hr), mp 161°.

Anal.—Calc. for $C_{13}H_9N_3O_5$: C, 54.36; H, 3.16; N, 14.63. Found: C, 54.73; H, 3.33; N, 14.48.

Determination of I as V or VI—*Direct Measurement of V or VI (Method 1)*—An exact volume (*e.g.*, 5, 10, 15, or 20 ml) of a standard ethanolic solution of I (*e.g.*, 2 × 10^{-5} *M*), prepared by diluting a standard stock solution (*e.g.*, 2 × 10^{-3} *M*), is mixed with a volume of the reagent solution (III or IV) in ethanol 1.5 times larger than that of the I but of the same concentration (*i.e.*, 2 × 10^{-5} *M*). (Equal volumes may also be used, but the concentration of the reagent is then 1.5 times that of I.) The mixture is evaporated under reduced pressure, and the residue is redissolved in a small amount of ethanol and reevaporated. This process is repeated for a third time, after which the colored product is dissolved in 5.0 ml of ethanol.

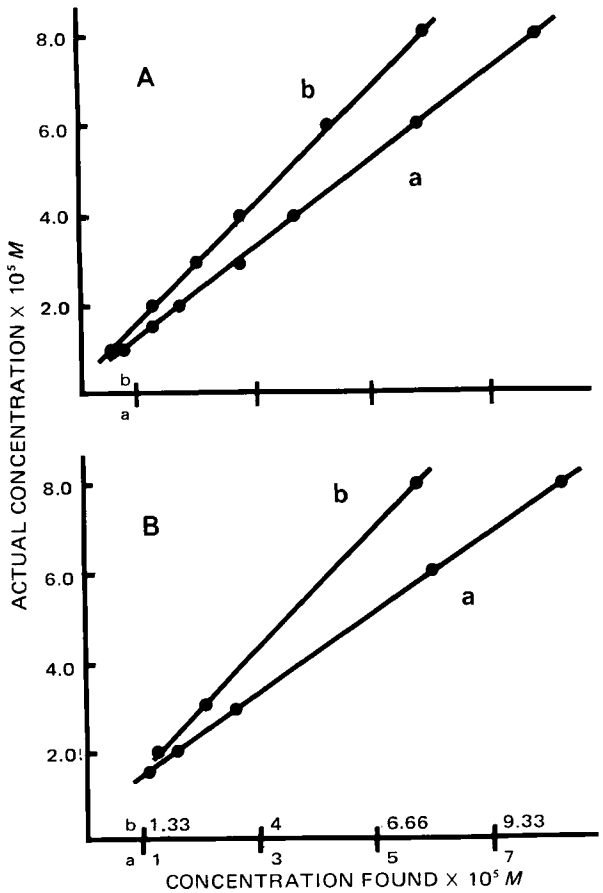
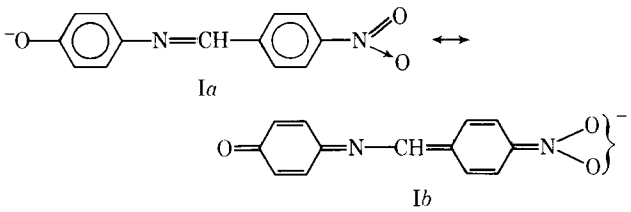


Figure 2—*Relationship between actual and determined (Method 3) I in excess of II. A: recovery of I calculated on the basis of the simultaneous analysis of reference samples of I. Key: a, I determined as V (slope = 0.97); and b, I determined as VI (slope = 0.98). B: recovery of I calculated on the basis of Beer–Lambert relationships using appropriate conversion factors. Key: a, I determined as V (slope = 0.91); and b, I determined as VI (slope = 1.02).*



Scheme I

Two milliliters of this solution is pipetted into a 1.0-cm silica cell and its spectrum is recorded. Absorbances read at the λ_{max} of 380 nm for V and of 404 nm for VI are multiplied by the appropriate conversion factors (7.23 × 10^{-5} and 9.58 × 10^{-5} for V and VI, respectively) derived from the Beer–Lambert linear relationships to convert them to concentrations of I. Then 0.5 ml of 0.1 *M* sodium hydroxide ethanolic solution is added to the cell and the spectra are recorded again. Absorbances are read at the λ_{max} of 466 and 528 nm, respectively.

Measurement of V or VI after Recovery by TLC (Method 2)—This method is applicable when degradation products are present. The concentrations of I and reagent solutions used are 10 times greater than those used in Method 1 (2 × 10^{-4} *M*). The same procedure is then followed until the reaction product is dissolved in 5.0 ml of ethanol. Then 0.5 ml of this final solution is spotted as a band on a TLC plate.

After elution, the yellow band corresponding to V or VI is scraped off and extracted with ethanol until the silica gel is free from any yellow color (filtration under gentle suction). The filtrate and washings are then evaporated under reduced pressure, and the reaction product is dissolved in 5.0 ml of ethanol. The spectra are then examined as outlined in Method 1. (Appropriate conversion factors are 8.16 × 10^{-5} and 10.4 × 10^{-5} for V and VI, respectively.)

Determination of I Present in II—This method also is applicable when degradation products are present.

Preparation of Samples—To a known amount of II (*e.g.*, 0.05, 0.5, or 5.0 g), an exact volume (*e.g.*, 2.5 or 7.5 ml) of a standard ethanolic I solution (2 × 10^{-3} *M*) is added and the resultant mixture is evaporated under reduced pressure.

Analysis of V or VI after Separation and Recovery by TLC from II (Method 3)—The solid mixture of I and II obtained after evaporation is extracted with 25 ml of water–alcohol (95:5 v/v) by shaking the suspension for about 0.5 hr. The suspension is filtered under gentle suction. Then an exact volume (5, 10, 15, or 20 ml) from the filtrate, whose pH is checked to be ~6, is mixed with a known volume of ethanolic III or IV solution calculated to be 1.5 times that of the I solution[9]. The resultant solutions are then evaporated to dryness three times under reduced pressure, and the reaction product is dissolved in a known volume of ethanol (5 ml). After 0.5 ml of this solution is spotted on the TLC plate, the same procedure as that described for Method 2 is then followed.

RESULTS AND DISCUSSION

Compound IV in ethanolic solution deteriorated more rapidly than III (about 50 and 6% in 5 days at ambient temperature, respectively), and the determination of I with deteriorated reagent solution was unsatisfactory. For example, after a solution of IV in ethanol stood for 3 weeks, it gave unsatisfactory analytical results. An aqueous solution of I deteriorated rapidly due to oxidative degradation, as evidenced by the formation of a dark precipitate and the analytical results using Method 2 (Table I). An alcoholic solution of I was much more stable in spite of the apparent color deterioration; less than 5% of I, determined by Method 2, was degraded after 1 week.

The absorbances of the neutral ethanolic solutions of V and VI were determined at λ_{max} 380 and 404 nm, respectively. A bathochromic shift took place when the solutions were made alkaline,

[9] When analyzing unknown mixtures, duplicate determinations with varying amounts of reagent solutions may be needed to establish that the reagent is in excess.

Table II—Stability of Neutral Ethanolic Solutions of V and VI[a]

	Compound V				Compound VI					
	6×10^{-5} M[b]		5×10^{-4} M[c]		6×10^{-5} M[b]		5×10^{-4} M[b]		5×10^{-4} M[c]	
Days	A	% Loss	A	% Loss	A	% Loss	A	% Loss	A	% Loss
0	0.870	—	0.748	—	0.699	—	0.578	—	0.580	—
1	0.752	13.6	0.401	46.4	0.688	1.6	0.567	1.9	0.486	16.3
2	0.636	26.9	0.396	47.1	0.668	4.4	0.560	3.1	0.444	23.5
4	0.421	51.6	—	—	0.632	9.6	0.550	4.8	0.400	31.0
5	0.370	57.5	0.378	49.5	0.603	13.7	0.540	6.6	0.360	34.1

[a] Compound V measured at 380 nm, and Compound VI measured at 404 nm. Absorbance in 1.0-cm silica cell. Absorbances were read from full UV–visible spectra. [b] Measurement of the absorbance before recovery by TLC. [c] Measurement of the absorbance after recovery by TLC.

Table III—Absorbance of Solutions of V in Ethanol in the Absence and in the Presence of Excess III

	4×10^{-5} M V	6×10^{-5} M V	8×10^{-5} M V	Mean Value	4×10^{-5} M V	4×10^{-5} M V and 4×10^{-5} M III	4×10^{-5} M V and 8×10^{-5} M III	Mean Value
No TLC[a]								
A_1[b] at 380 nm	0.578	0.868	1.16	—	0.577	0.580	0.566	0.574
A_2 at 466 nm	0.710	1.07	1.42	—	0.720	0.721	0.696	0.712
Ratio A_2/A_1	1.23	1.23	1.22	1.23	1.24	1.24	1.23	1.24
TLC[c]								
A_1 at 380 nm	0.491	0.759	1.01	—	0.491	0.522	0.520	0.512
A_2 at 466 nm	0.606	0.934	1.25	—	0.606	0.658	0.658	0.641
Ratio A_2/A_1	1.24	1.23	1.24	1.24	1.23	1.26	1.26	1.25
Percent recovered at 380 nm	84.9	87.4	87.1	86.5	85.0	90.0	91.9	90.1
Percent recovered at 466 nm	85.3	87.5	87.9	86.9	84.2	91.3	94.5	91.3

[a] Measurement of absorbance before recovery by TLC. [b] Absorbance in 1.0-cm silica cell. [c] Measurement of absorbance after recovery by TLC.

Table IV—Absorbance of Solutions of VI in Ethanol in the Absence and in the Presence of Excess IV

	4×10^{-5} M VI	6×10^{-5} M VI	8×10^{-5} M VI	Mean Value	4×10^{-5} M VI	4×10^{-5} M VI and 4×10^{-5} M IV	4×10^{-5} M VI and 8×10^{-5} M IV	Mean Value
No TLC[a]								
A_1[b] at 404 nm	0.465	0.693	0.925	—	0.465	0.443	0.448	0.452
A_2 at 528 nm	0.710	1.08	1.42	—	0.720	0.687	0.685	0.697
Ratio A_2/A_1	1.53	1.56	1.54	1.54	1.55	1.55	1.53	1.54
TLC[c]								
A_1 at 404 nm	0.427	0.640	0.884	—	0.427	0.429	0.427	0.428
A_2 at 528 nm	0.649	0.934	1.27	—	0.649	0.652	0.653	0.651
Ratio A_2/A_1	1.52	1.46	1.44	1.46	1.52	1.52	1.53	1.52
Percent recovered at 404 nm	91.8	92.3	95.6	93.3	91.8	96.8	95.3	94.6
Percent recovered at 528 nm	91.4	87.8	89.8	89.4	90.1	94.9	95.2	93.4

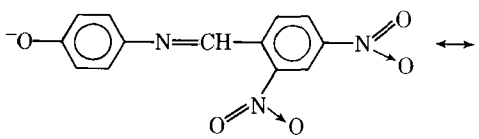
[a] Measurement of absorbance before recovery by TLC. [b] Absorbance in 1.0-cm silica cell. [c] Measurement of absorbance after recovery by TLC.

and the absorbance could then be read at 466 and 528 nm, respectively. The bathochromic shift was due presumably to resonance between Ia and Ib and IIa and IIb (Schemes I and II). Not only the alkaline but the neutral solutions of V and VI were unstable. Recovery of these products by TLC from standing solutions also showed losses which increased with standing time (Table II).

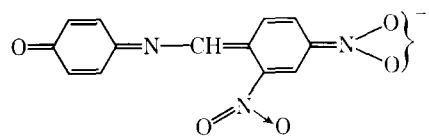
Freshly prepared solutions, however, obeyed the Beer–Lambert relationship. After recovery by TLC (Tables III and IV), the results were lower by an average of 12% when I was determined as V and 8% when I was determined as VI, but they still gave a satisfactroy Beer–Lambert relationship. Excess reagent had no effect on the absorbance readings (Tables III and IV).

It is more accurate to construct a Beer–Lambert relationship by using standard ethanolic solutions of I (Tables V and VI). The direct reaction of I with the reagent solutions as described in Method 1 (not using TLC) gave results that were 3.3 or 9.5% lower than those obtained by measuring directly pure V or VI, respectively. The direct reaction of I with reagent solutions as described in Method 2 (using TLC) gave results that were 1.0 or 11.0% lower than those obtained for pure V or VI, respectively. These data suggest that the decrease in the results due to the direct reaction of I with the reagent solutions before and after recovery by TLC is rather constant. The average values for this decrease were 2.2 or 10.3% for V or VI, respectively. Moreover, a large excess of reagent was unnecessary for the reaction to go to completion (Tables V and VI).

It is also possible to calculate the decrease in the results due to losses occurring during the use of TLC. Thus, these losses for pure



IIa



IIb

Scheme II

Table V—Absorbance of Ethanolic Reaction Solutions of I with III

	4×10^{-5} *M* I and 6×10^{-5} *M* III	6×10^{-5} *M* I and 9×10^{-5} *M* III	8×10^{-5} *M* I and 12×10^{-5} *M* III	Mean Value
No TLC[a]				
A_1[b] at 380 nm	0.552	0.840	1.10	—
A_2 at 466 nm	0.671	1.03	1.37	—
Ratio A_2/A_1	1.22	1.23	1.24	1.23
TLC[c]				
A_1 at 380 nm	0.477	0.740	0.985	—
A_2 at 466 nm	0.581	0.910	1.20	—
Ratio A_2/A_1	1.22	1.23	1.22	1.22
Percent recovered at 380 nm	86.4	88.1	89.5	88.1
Percent recovered at 466 nm	86.6	88.1	87.7	88.8

[a] Measurement of absorbance before recovery by TLC. [b] Absorbance in 1.0-cm silica cell. [c] Measurement of absorbance after recovery by TLC.

V and VI were 13.5 and 6.5%, respectively, while those for the direct reaction of I with the reagent solutions were 11.5 and 8.0%, respectively. The agreement between these figures is satisfactory when it is considered that the experimental error of the present method is about ±5%. Therefore, the average value for the losses due to TLC was 12.5 or 7.5% for the determination of I as V or VI, respectively.

A correction factor to account for losses due to TLC is not needed to convert absorbances to concentrations when the appropriate Beer–Lambert linear relationship is used. However, if a Beer–Lambert relationship is constructed using only pure V or VI as reference materials, correction factors are needed. The average factor calculated for this case is 1.17 or 1.21 for the determination of I as V or VI, respectively. On the other hand, if a Beer–Lambert relationship is constructed by the direct reaction of I with the reagent solutions of III or IV, then the average correction factor needed to correct for losses due to the recovery by TLC is 1.14 or 1.08 for the determination of I as V or VI, respectively. Nonetheless, it would be preferable to construct and use the appropriate Beer–Lambert linear relationship in each case.

The ratios of the absorbance of the alkaline solutions to that of the neutral solutions of V and VI were 1.24 and 1.52, respectively, when no TLC was used. After recovery by TLC, however, there was a small tendency for the ratios to decrease, especially for VI (Tables IV and VI). These ratios can be used as an additional check of the correct identification and accurate determination of V and VI. The percentage recovery figures were calculated from the readings obtained only at the λ_{max} of the neutral solutions, *i.e.*, 380 and 404 nm for V and VI, respectively.

A number of samples of II containing various amounts of I (0.05–4.2%) were analyzed by following Method 3, and the results were compared with those obtained with pure reference samples of I. These reference samples were analyzed under identical conditions and used as the basis for the percentage calculation of the recovery results (Table VII). These results were also compared with those obtained by a modification of Method 3, which seems satisfactory for an approximate determination of I in II, especially for samples not degraded. This procedure consisted of reading the absorbances after carrying out the appropriate dilutions and omitting the use of TLC for separating V or VI from the mixture. When TLC separation was used, the recovery was almost 100% for mixtures containing more than 0.3% of I; for mixtures containing less than 0.3%, the recovery figures decreased with a decrease in the I content, presumably due to some retention by the great excess of II.

The present method gave reproducible results (Tables VIII and IX) for mixtures also containing very small amounts of I and for

Table VI—Absorbance of Ethanolic Reaction Solutions of I with IV

	4×10^{-5} *M* I and 6 $\times 10^{-5}$ *M* IV	8×10^{-5} *M* I and 12 $\times 10^{-5}$ *M* IV	Mean Value	6×10^{-5} *M* I and 12 $\times 10^{-5}$ *M* IV	6×10^{-5} *M* I and 18 $\times 10^{-5}$ *M* IV	Mean Value
No TLC[a]						
A_1[b] at 404 nm	0.411	0.822	—	0.632	0.626	—
A_2 at 528 nm	0.625	1.25	—	0.971	0.971	—
Ratio A_2/A_1	1.52	1.52	1.52	1.54	1.55	1.55
TLC[c]						
A_1 at 404 nm	0.396	0.774	—	0.588	0.605	—
A_2 at 528 nm	0.575	1.15	—	0.853	0.859	—
Ratio A_2/A_1	1.45	1.49	1.47	1.45	1.42	1.44
Percent recovered at 404 nm	96.4	94.2	95.3	93.0	96.6	94.8
Percent recovered at 528 nm	92.0	92.0	92.0	87.8	88.6	88.2

[a] Measurement of absorbance before recovery by TLC. [b] Absorbance in 1.0-cm silica cell. [c] Measurement of absorbance after recovery by TLC.

Table VII—Recovery of I in Laboratory Prepared Samples of II Containing Known Amounts of I[a]

		Compound V			Compound VI		
I, mg	II, mg	% Recovery[b], CF[d]	% Recovery[b], RS[e]	% Recovery[c], RS[e]	% Recovery[b], CF[d]	% Recovery[b], RS[e]	% Recovery[c], RS[e]
0.275	500	—	75.3	82.5	—	76.9[f]	85.2[f]
0.410	500	75.6	82.7	92.4	—	—	—
0.545	500	78.8	84.6[g]	93.3[g]	84.2	81.8[g]	93.0[g]
0.820	500	86.2	89.3	93.4	93.9	88.3	95.2
1.090	500	—	92.0[h]	96.9[h]	91.3	91.5[h]	98.9[h]
1.635	500	100.4	96.5	97.2	—	94.9	—
2.180	500	102.4	98.3	98.9	94.6	99.3	—
0.545	50	95.8	99.6	98.3	97.2	105.0	—
1.090	50	97.3	100.0	100.0	104.3	98.3	—
1.635	50	95.5	96.8	101.2	105.7	101.2	—
2.180	50	102.4	99.6	98.6	105.9	102.5	—

[a] Compound V measured at 380 nm, and Compound VI measured at 404 nm. Absorbances were read from full UV–visible spectra. [b] Measurement of absorbance after recovery by TLC. [c] Measurement of absorbance before recovery by TLC. [d] Calculation of percent recovery using conversion factors 8.16×10^{-5} and 10.4 for V and VI, respectively. [e] Calculation of percent recovery on the basis of simultaneous analysis of reference samples of I. [f] Mean value of three determinations. [g] Mean value of two determinations. [h] Mean value of five determinations.

Table VIII—Reproducibility of Results[a]

I, mg	II, mg	Compound V		Compound VI	
		% Recovery, TLC	% Recovery, No TLC	% Recovery, TLC	% Recovery, No TLC
0.273	500	75.3		75.6	83.8
0.273	500	— } 75.3[b]		75.9 } 76.9	84.1 } 85.2
0.273	500	—		79.2	87.7
0.273	—			100.0	100.0
0.55	500	85.3 } 84.6	93.6 } 93.3	82.0 } 81.8	92.8 } 93.0
0.55	500	83.9	93.0	81.6	93.2
0.55	—	100.0	100.0	100.0	100.0
1.09	500	93.2	98.2	88.9	95.2
1.09	500	91.7	96.5	92.6	96.2
1.09	500	90.8 } 92.0	96.6 } 96.9	93.4 } 91.5	102.5 } 98.9
1.09	500	91.3	96.9	92.3	98.7
1.09	500	93.0	96.4	90.4	101.8
1.09	—	100.0	100.0	100.0	100.0

[a] Compound V measured at 380 nm, and Compound VI measured at 404 nm. Absorbances were read from full UV–visible spectra. [b] Mean value.

which the recovery figures were about 80%. These results suggest that laboratory samples of II containing a known amount of I also can be used as reference material for analyzing unknown mixtures.

The following solvent mixtures were unsatisfactory for the recovery of the Schiff bases by TLC, because V and VI hydrolyzed in these systems: (*a*) chloroform–acetone–acetic acid (80:18:2), (*b*) chloroform–petroleum ether–acetone (70:20:10), and (*c*) chloroform–petroleum ether (80:20). Hydrolysis was faster in Solvent System (*a*), especially for V (almost complete).

Dark degradation products of I do not interfere with the determination of I by Method 2 or 3 because the solvent system [petroleum ether–acetone (70:30 v/v)] separates V or VI from the reaction mixtures (Fig. 1 and Table IX). This finding was confirmed for all TLC separations when full UV–visible spectra of the components separated and then recovered by extraction of the silica gel with alcohol were recorded. These spectra were compared with those of pure samples of the various components to ascertain identity, as, for example, for samples of II, V, or VI. Incomplete separation can be established by such a comparison. Thus, when petroleum ether–acetone (20:80 v/v) was used as the solvent system for the TLC recovery during the analysis of a degraded sample of I, the spectrum of the yellow band corresponding to V was different from that of the authentic material; *i.e.*, in neutral solution the λ_{max} was 402 nm instead of 380 nm and in alkaline solution it was 510 nm instead of 466 nm. Proper TLC separation then confirmed that the yellow band was a mixture of more than one component.

In conclusion, it is recommended that samples of I can be analyzed as V or VI by using Method 1 or 3, depending on whether I is degraded or not. If I is present in II in amounts exceeding 0.3%, it is sufficient to use the appropriate conversion factors to calculate the concentration of I from the final absorbances. If the amount of I in II is less than 0.3%, it is possible to use a graphical relationship derived from the results of Table VII (Fig. 2) of the actual amount of I against the amount determined by Method 3 and calculated directly by using the appropriate conversion factors or by using the results of analysis of pure reference samples of I as the basis of comparison. However, for each analysis of an unknown sample, a reference sample of II containing a known amount of I should be analyzed also for comparison.

The use of an appropriate densitometer for the determination of all separated products directly on the same TLC plate could increase the speed and accuracy of the method and, perhaps, the detection limits.

Table IX—Recovery of I in Deteriorated Samples of II and Reproducibility of Results[a]

I, mg	II, mg	Percent Recovery of V, TLC	Percent Recovery of VI, TLC
1.09	500	95.8	92.2
1.09	500	94.3 } 95.7[b]	94.0 } 93.6
1.09	500	96.9	94.5
—	500	4.0	5.2
1.09	—	100.0	100.0

[a] Compound V measured at 380 nm, and Compound VI measured at 404 nm. Absorbances were read from full UV-visible spectra. [b] Mean value.

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