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5-(*p*-Dimethylaminophenyl)-2,4-pentadienal as an Analytical Reagent: A Simple Preparation of the Reagent and Its Application to the Colorimetric Determination of Primary Aromatic Amines

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5-(*p*-Dimethylaminophenyl)-2,4-pentadienal (DAPDA) was conveniently prepared from *p*-dimethylaminobenzaldehyde by using readily available 4-(*tert*-butylthio)-3-buten-2-one as the chain lengthening agent. It has been shown that DAPDA is a useful reagent for detection and colorimetric determination of many pharmaceuticals (*e.g.*, ethyl *p*-aminobenzoate (EAB), procaine hydrochloride, sulfisoxazole, sulfamethoxazole, sodium *p*-aminosalicylate, *etc.*) containing primary aromatic amino groups. This method is based on the reaction of these amines with DAPDA in the presence of trichloroacetic acid in methanol to form protonated Schiff bases which can be determined spectrophotometrically in the neighborhood of 630 nm. The limit of detection of these aromatic amines was in the range of 0.05—1.0 μg/ml in each case. Lambert–Beer's law holds over 0.2—10 μg/ml. The results were comparable to those obtained by hitherto reported methods. The method was successfully applied to commercial preparations of primary aromatic amines.

Keywords——4-(*tert*-butylthio)-3-buten-2-one; $\alpha,\beta,\gamma,\delta$-unsaturated aldehyde; 5-(*p*-dimethylaminophenyl)-2,4-pentadienal; synthon; sulfanylamide analysis; colorimetric reagnt for primary aromatic amine; primary aromatic amine; protonated Schiff base; colorimetry of primary aromatic amine

In the previous papers, 4-(*tert*-butythio)-3-buten-2-one(I)[1a-c] has been proved to be a convenient synthon for $\alpha,\beta,\gamma,\delta$-unsaturated aldehydes and for the construction of important synthetic intermediates of annulenoannulenes[2] and isoprenoid polyene chains, *e.g.*, isorenieratene.[1b] We wish to report here a facile preparation of 5-(*p*-dimethylaminophenyl)-2,4-pentadienal[*p*-dimethylaminophenylpentadienal(DAPDA)], a higher vinylog of Ehrlich's reagent (II),[3] using I and its application to the detection and the colorimetric determination of several pharmaceuticals containing aromatic primary amino groups. It was found that DAPDA could readily condense with aromatic primary amines in acidic methanol solution to show a deep blue color. Consequently, we have fully examined a simple and rapid detection by coloration and determination of several aminobenzene derivatives in the longer wavelength region. As described later, the optimal experimental conditions for detection and determination of various primary aromatic amines have been established successfully.

Experimental

Apparatus——Absorption at a fixed wavelength was measured with a Shimadzu 150 double beam spectrophotometer. Absorbance at various wavelengths was recorded with a Shimadzu ultraviolet (UV) 300 spectrophotometer Matched cells of 10 mm path length were used. Infrared (IR) spectra were measured with a JASCO IRA-2 spectrometer and ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL FX 90Q spectrometer with tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were taken with a JEOL JMA-01SG machine. All melting points are uncorrected.

Reagents and Solvents——Aromatic amines for the detection test and for the determination were used after recrystallization from ethanol or/and ether. Guaranteed reagent grade trichloroacetic acid (TCA) was purchased from Ishizu Pharmaceutical Co. Ltd., Tokyo. All other reagents used were of reagent grade. A 50% (w/v) solution of TCA in MeOH was prepared just before use. A stock solution[4] of DAPDA (0.1%

(w/v)) in MeOH was prepared for the determination of the aromatic primary amines. Dotite Spectrosol MeOH (Dojin, Kumamoto) was used. All other solvents used were purified by distillation.

1-*tert*-Butylthio-5-p-dimethylaminophenylpenta-1,4-dien-3-one (III)——A solution of NaOH (3.6 g, 90 mmol) in water–ethanol (4: 1, 5 ml) was added dropwise to a solution of *p*-dimethylaminobenzaldehyde (II) (3.00 g, 19 mmol) and 4-(*tert*-butylthio)-3-buten-2-one (I)1) (4.5 g, 28 mmol) in EtOH (10 ml). After 2 h, crystals that had deposited were filtered off, washed with EtOH–H_2O, and dried *in vacuo*. The product was recrystallized from ether–CH_2Cl_2 and washed with hexane to give III as orange crystals (3.84 g, 66% from II), mp 116—118°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: 1620 (C=O), 1550 (C=C). MS *m/e*: 289 (M^+). NMR ($CDCl_3$, δ): 1.46 (9H, s, *tert*-Bu), 3.00 (6H, s, Me_2N), 6.60 (1H, d, $J_{cd}=15$ Hz, H^c), 6.63 (1H, $J_{ef}=9$ Hz, H^f), 7.41 (1H, $J_{ef}=9$ Hz, H^e), 7.55 (1H, d, $J_{cd}=15$ Hz, H^d), 7.75 (1H, d, $J_{ab}=15$ Hz, H^b), 7.93 (1H, d, $J_{ab}=15$ Hz, H^a). *Anal.* Calcd for $C_{17}H_{23}NOS$: C, 70.59; H, 7.96; N, 4.84. Found: C, 70.64; H, 8.08; N, 4.78.

5-*p*-Dimethylaminophenylpenta-2,4-dien-1-al (DAPDA)——To a solution of III (4.00 g, 14 mmol) in ether (200 ml) was added at room temperature a stock solution of $LiAlH_4$ (0.07 M, 200 ml, equivalent to III) in the same solvent, and the mixture was kept for 1 h at the same temperature. Dilute sulfuric acid (2 M, 200 ml) was added to the reaction mixture in an ice bath. After being stirred for 3 h, the mixture was neutralized with NaOH (4 M), and then extracted with ether. The extract was worked up to yield DAPDA as light brown crystals (2.65 g, 94%), mp 150—151°C (lit.,5) mp 153—155°C). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: 1665 (C=O), 1588 (C=C). NMR ($CDCl_3$, δ): 3.00 (6H, s, NMe_2), 6.13 (1H, $J_{ab}=8$ Hz, $J_{bc}=15$ Hz, H^b), 6.70 (2H, $J_{fg}=9$ Hz, H^g), 6.74—7.21 (3H, m, H^e, H^d, and H^e), 7.35 (2H, $J_{fg}=9$ Hz, H^f), 9.49 (1H, d, $J_{ab}=8$ Hz, H^a). MS *m/e*: 201 (M^+). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ε): 413 (4.35). *Anal.* Calcd for $C_{13}H_{15}NO$: C, 77.61; H, 7.46; N, 6.97. Found: C, 77.45; H, 7.64; N, 6.74.

Examination of Detection Limit——A test solution (50 μl) of known concentration in MeOH was pipetted into a glass-stoppered test tube (1 ml), then 0.1% (w/v) DAPDA in MeOH (50 μl) and 50% (w/v) TCA in MeOH (50 μl) were added successively. The detection limit data are summarized in Table I.

Thin Layer Chromatographic (TLC) Analysis——TLC of some primary aromatic amines was carried out on glass plates (20×20 cm, 0.25 mm thick) coated with Kieselgel 60 (Merck), with three different solvent systems. The chromatogram obtained was treated with DAPDA (0.1% (w/v)) in TCA–MeOH (50% v/v)) and the *Rf* values were determined, as shown in Table II.

Preparation of the Calibration Curve——A standard solution was made by diluting the sample (80.0—100.0 mg) with MeOH to 100 ml; a definite volume was pipetted out for dilution to each desired concentration (2—100 μg/ml) with MeOH.

Recommended Procedure——To a sample solution (1 ml, 2—100 μg/ml) in a 10 ml volumetric flask, 0.1% (w/v) DAPDA–MeOH solution (1.0 ml) and 50% (w/v) TCA–MeOH solution (1.0 ml) are added, and the whole is diluted to 10 ml with MeOH. The solution is mixed and allowed to stand for 10 min, then the absorbance is measured at the appropriate wavelength against the reagent blank.

Determination of Recovery——The preparations (powders) (50.0—250.0 mg) were dissolved in MeOH, and the injections and eye lotions were diluted with to contain the concentration levels denoted in Table IV. Each sample solution (1.0 ml) was pipetted into a 10 ml volumetric flask and assay was carried out according to the recommended procedure. The contents were estimated from the calibration curves obtained with the standard sample solutions.

Results and Discussion

Synthesis of DAPDA

As shown in Chart 1, the aldol condensation of II with I in the presence of NaOH gave thiovinyl ketone (III) (66% from II), which was reduced with $LiAlH_4$ in ether to give the alcohol (IV). The alcohol (IV) was treated without isolation with 2 M sulfuric acid. After neutralization, DAPDA was obtained in high yield (94%).

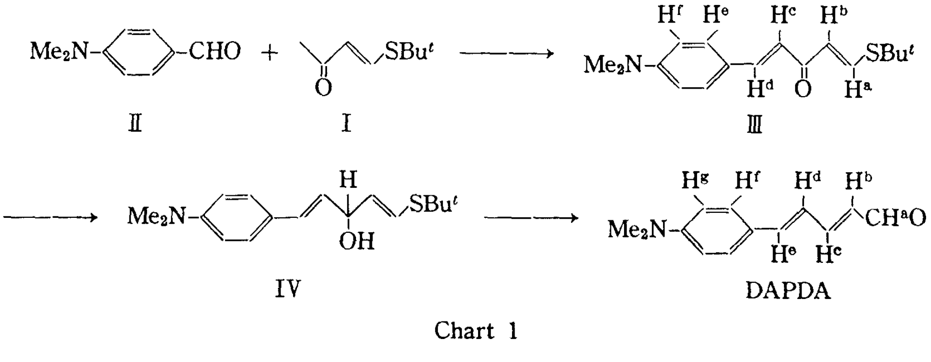

Chart 1

Application of DAPDA to Analysis of Primary Aromatic Amines

Primary aromatic amines could condense quite easily with DAPDA in an acidic MeOH solution to produce intensely colored protonated Schiff bases, which seemed to result from the formation of a charged resonance system as illustrated in Chart 2. Although the formation of such a protonated Schiff base is already known for Ehrlich's reagent[6] and its vinylog [*p*-dimethylaminocinnamaldehyde (DACA)],[6,7] that of DAPDA has a characteristically intense absorption maximum [628 nm for ethyl *p*-aminobenzoate (EAB)] in a much longer-wavelength region than those of the lower vinylogs (*ca.* 430[6] and *ca.* 530 nm,[7] respectively).

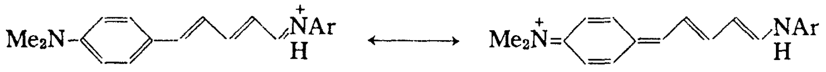

Chart 2

The easy formation of a protonated Shiff base with DAPDA could be applied to the drop analysis of a variety of aromatic primary amines. As shown in Table I a number of amines were colored and examined to determine the detection limit[8] (0.05—1.0 μg); however, in the case of *o*-nitroaniline, its steric hindrance was clearly reflected in the reaction.

Table I. Limits of Detection of Primary Aromatic Amines

Amine	Amount of amines (μg)										Color
	50.0	25.0	5.0	2.0	1.0	0.5	0.2	0.05	0.02	0	
Aniline	+	+	+	+	+	+	+	±	−	−	Bluish-violet
p-Aminobenzoic acid	+	+	+	+	+	+	±	−	−	−	Blue
m-Nitroaniline	+	+	+	+	+	+	±	−	−	−	Blue
o-Phenylenediamine	+	+	+	+	+	+	±	−	−	−	Brown
p-Aminosalicylic acid	+	+	+	+	+	+	±	−	−	−	Blue
Sulfanylamide	+	+	+	+	+	+	±	−	−	−	Blue
EAB	+	+	+	+	+	+	±	−	−	−	Blue
Sulfisoxazole	+	+	+	+	+	+	±	−	−	−	Blue
Sulfamethoxazole	+	+	+	+	+	+	±	−	−	−	Blue
Procaine Hydrochloride	+	+	+	+	+	+	±	−	−	−	Blue
m-Aminophenol	+	+	+	+	+	±	−	−	−	−	Dark blue
p-Nitroaniline	+	+	+	+	+	±	−	−	−	−	Bluish-green
p-Nitro-*o*-aminophenol	+	+	+	+	+	±	−	−	−	−	Blue
p-Nitro-*o*-anisidine	+	+	+	+	+	±	−	−	−	−	Blue
o-Tolidine	+	+	+	+	+	±	−	−	−	−	Blue
p-Nitro-*o*-toluidine	+	+	+	+	±	−	−	−	−	−	Blue
o-Nitroaniline	−	−	−	−	−	−	−	−	−	−	—

+ : positive, − : negative, ± : detection limit.

This coloration reaction was positive with secondary aromatic amines, certain heteroaromatics (*e.g.*, pyrrole, indole), and urea derivatives including barbiturates. It is of interest that the above reaction was negative to aliphatic amines and active methylene compounds (*e.g.*, acetone, creatinine, estrone, or dehydrocholic acid), in contrast to the case of Ehrlich's reagent. Aromatic primary amines could be analyzed qualitatively by TLC. The colors on the plates and the *Rf* values determined are summarized in Table II.

As regards the colorimetric determination of primary aromatic amines, the determination conditions for EAB are mentioned below as a typical example. The absorption spectra of EAB (20 μg/ml) in the mixture of DAPDA-MeOH (0.1% (w/v)) and in TCA-MeOH (50% (w/v)) solution are shown in Fig. 1. Determination is done at the absorption maximum against the reagent blank similarly prepared but free from EAB. As illustrated in Fig. 2, a nearly constant absorbance was obtained in the concentration range of 20—80% (w/v) TCA in

Table II. TLC of Primary Aromatic Amines

Amine	*Rf* values[a] A[b]	*Rf* values[a] B[c]	*Rf* values[a] C[d]	Color[e]
m-Aminophenol	0.65	0.54	0.22	BV
p-Aminobenzoic acid	0.71	0.39	0.15	BG
Aniline	0.83	0.83	0.69	BV
p-Nitro-*o*-toluidine	0.84	0.87	0.71	B
p-Nitroaniline	0.78	0.75	0.45	BG
m-Nitroaniline	0.84	0.82	0.60	B
o-Phenylenediamine	0.30	0.57	0.27	BV
p-Nitro-*o*-aminophenol	0.81	0.59	0.24	DB
p-Nitro-*o*-anisidine	0.84	0.90	0.76	DB
o-Tolidine	0.67	0.81	0.48	BV
Sulfanilamide	0.38	0.29	0.07	B
EAB	0.82	0.81	0.61	B
Sulfisoxazole	0.51	0.33	0.10	B
Sulfamethoxazole	0.61	0.51	0.16	B
p-Aminosalicylic acid	0.59	0.10	0.04	B
Procaine hydrochloride	0.09	0.31	0.12	B

TLC plates were dried at 110°C for 30 min just before use.

a) The mean of triplicate assays.

b) Ether. *c*) Chloroform: MeOH=10: 1. *d*) Benzene: MeOH=9: 1.

e) BV=bluish-violet; BG=bluish-green; B=blue; DB=dark blue.

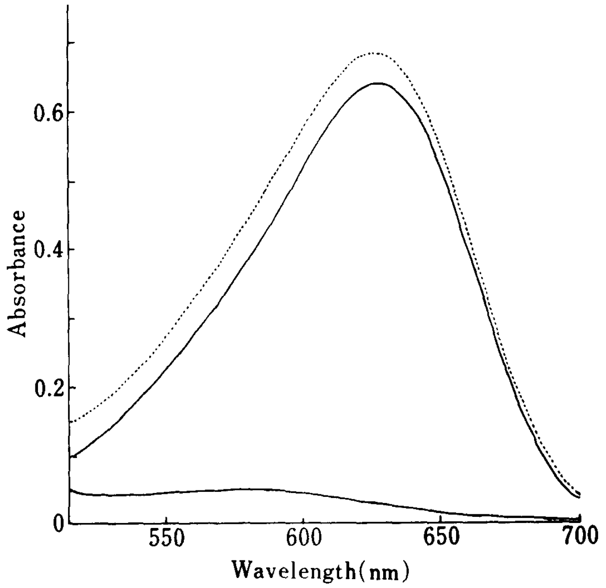

Fig. 1. Absorption Spectra

I: reaction mixture of EAB against reagent blank.
II: reaction mixture of EAB against MeOH.
III: reagent blank against MeOH.

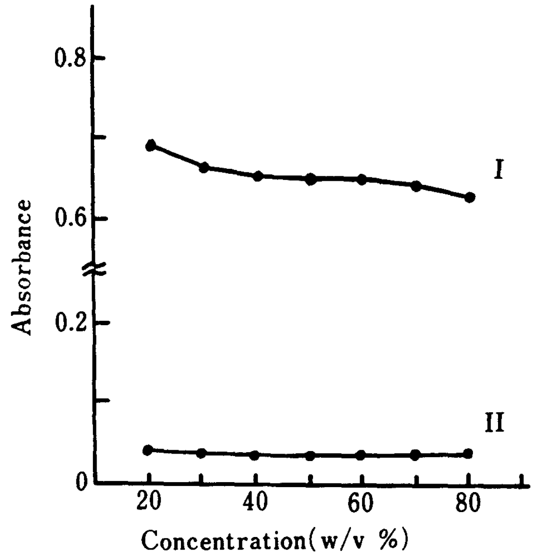

Fig. 2. Effect of Concentration of TCA on the Absorbance at 628 nm (EAB: 20 μg/ml)

I: protonated Schiff base of EAB against reagent blank.
II: reagent blank against MeOH.

MeOH. We decided to use a 50% concentration of the acid for the assay. The effect of DAPDA concentration in MeOH (from 0.05 to 0.25% (w/v) on the absorption change with EAB (20 μg/ml) when all other conditions were held constant was checked. The results are shown in Fig. 3, from which the optimum reagent concentration was found to be 0.1% (w/v). The color development was immediate, and resulting color was stable for at least 40 min, as shown in Fig. 4.

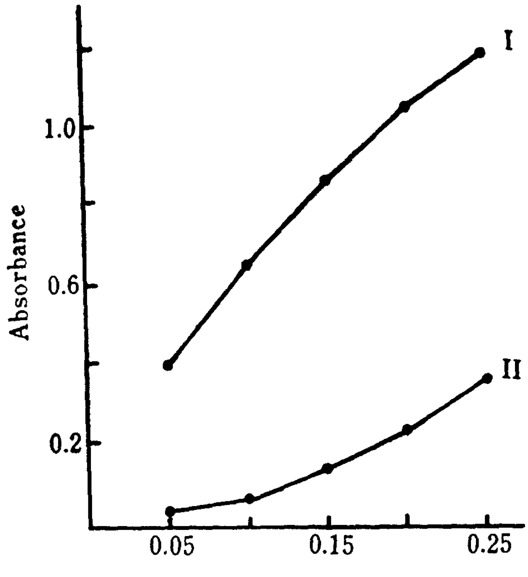

Fig. 3. Effect of Concentration of DAPDA on the Absorbance at 628 nm (EAB: 20 μg)

I: protonated Schiff base of EAB against reagent blank.
II: reagent blank against MeOH.

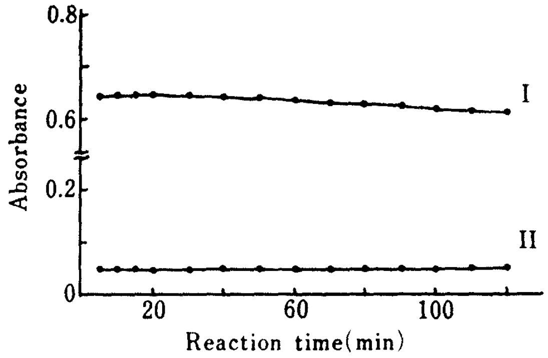

Fig. 4. Relation between Reaction Time and Absorbance at 628 nm (EAB: 20 μg/ml)

I: protonated Schiff base of EAB against reagent blank.
II: reagent blank against MeOH.

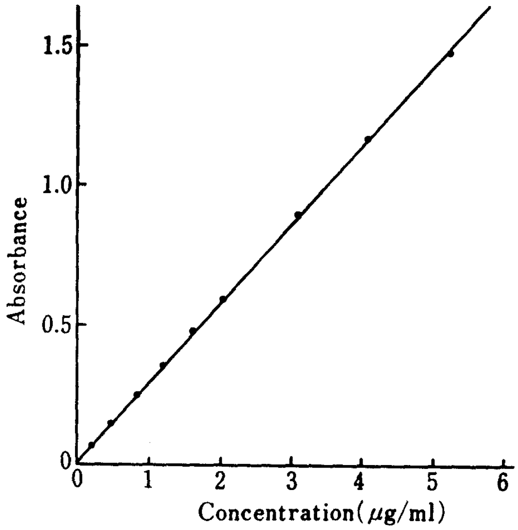

Fig. 5. Calibration Curve for EAB measured at 628 nm

We adopted a reaction time of 10 min for the determination. Various kinds of solvent, such as MeOH, EtOH, dimethylsulfoxide (DMSO), dimethylformamide (DMF), and tetrahydrofuran (THF), were examined using EAB (12 μg/ml) and DAPDA (0.1% (w/v)) for the coloration. Maximum intensity was obtained with MeOH or EtOH as the diluent. MeOH was superior to EtOH in terms of both the reagent blank value and the solubility of DAPDA. Consequently, MeOH was chosen as the diluent. The addition of water resulted in a decrease of absorbance, *e.g.*, in the case of using 20 μg/ml of the sample, 0.1, 0.5, 1, and 5% (v/v) water content gave 96, 93, 88, and 61% of the original value, respectively. Hydrochloric acid was inferior to TCA in the coloration reaction. The calibration curve for EAB was linear from 0.2 to 4.0 μg/ml, as shown in Fig. 5. The apparent molar absorptivity of the protonated Schiff base of EAB as estimated from the calibration curve was 5.25×10^4 $l \cdot mol^{-1} \cdot cm^{-1}$ (see Table III). The standard deviation and the coefficient of variation for the nine replicate sample solutions using EAB (20 μg/ml) were 0.005 and 0.78%, respectively.

Determination of Commercial Preparations

The colorimetric determination of commercial preparations (powders), *e.g.*, procaine hydrochloride, sulfisoxazole, sulfamethoxazole, and sodium *p*-aminosalicylate could be carried out satisfactorily under the same conditions as used for EAB, as shown in Table IV. The recovery was good (99.6—104.2%). In the cases of commercial solutions (injection and eye lotion) the determination results were also satisfactory as shown in Table V.

The diazotization titration method[9] used widely for the determination of aromatic primary amines with standard sodium nitrite at low temperature and starch-zinc iodide paper as the external indicator is subjective as regards determining the end-point. The principal advantages of our determination method are that it is simple, rapid, and sensitive. It permits analysis of a trace amount of the amines with reasonable accuracy and precision.

TABLE III. Absorption Maxima, Apparent Molar Absorptivities and Determination Ranges of Aminobenzene Derivatives

Sample	λ_{max}	ε_{app}	Determination range (µg/ml)
EAB	628	5.25×10^4	0.2—4.0
Procaine hydrochloride	630	2.89×10^4	0.5—9.0
Sulfisoxazole	633	2.58×10^4	0.5—10.0
Sulfamethoxazole	633	2.71×10^4	0.5—10.0
Sodium *p*-aminosalicylate	630	5.16×10^4	0.2—4.0

TABLE IV. Assay of Known Concentrations of Commercial Preparations

Sample	Amount weighed (mg)	Concentration (µg/ml)	Amount found (mg)	Percent recovery
Ethyl *p*-aminobenzoate	50.0	10	50.5	101.0
		25	50.2	100.4
	250.0	5	259.6	103.8
		30	249.1	99.6
Procaine hydrochloride	50.0	20	51.7	103.4
		50	49.8	99.6
	250.0	10	247.8	99.1
		60	252.9	101.2
Sulfisoxazole	50.0	20	49.5	98.9
		50	49.2	98.4
	250.0	10	253.8	101.5
		60	248.7	99.5
Sulfamethoxazole	50.0	20	52.1	104.2
		50	50.9	101.9
	250.0	10	245.3	98.1
		60	247.7	99.1
Sodium *p*-aminosalicylate	100.0	20	103.1	103.1

Each value is the average of triplicate assays.

TABLE V. Assay of Commercial Preparations (Solutions)

Commercial preparation[a]	Amount calculated (µg/ml)	Amount found (µg/ml)	Percent recovery
1% procaine hydrochloride	10.0	10.29	102.9
2% procaine hydrochloride	20.0	20.67	103.4
10% sulfisoxazole	100.0	98.95	99.0
20% sulfisoxazole	200.0	203.59	101.8
4% sulfamethoxazole	40.0	39.72	99.3

Each value is the average of triplicate assays.
[a] w/v percentage.

The sensitivity of the proposed method is superior to that of the colorimetry based on Bratton–Marshall's[10] and Tsuda's[11] diazotization method and is comparable to that obtained by the use of Ehrlich's reagent[12] and its vinylog.[13] However, several fluorometric procedures[14] are superior to our method in this respect.

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