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**SPECTROPHOTOMETRIC AND  
FLUORIMETRIC DETERMINATION  
OF AMINES**

*Prepared for publication by*

J. BARTOS and M. PESEZ

Roussel-Uclaf, F-93230 Romainville, France

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†Deceased.

## SPECTROPHOTOMETRIC AND FLUORIMETRIC DETERMINATION OF AMINES

Jaroslav Bartos and Maurice Pesez

Roussel-Uclaf F-93230 Romainville, France

**Abstract** - Thirteen procedures are described in this report for the colorimetric and fluorimetric determination of amines. In the presence of an appropriate base, 1,3,5-trinitrobenzene condenses with nitromethane to give a red Meisenheimer-type complex which allows determination of alkylamines and quaternary ammoniums. The mobility of the hydrogen atom (or atoms) bonded to the amino nitrogen atom of primary and secondary alkyl- and arylamines, allows derivatives which permit general or selective determinations. Primary and secondary alkyl- and arylamines are so estimated through the formation of N-substituted derivatives of p-nitrophenylazobenzamide or of 2,4-dinitroaniline (according to another procedure, only primary alkylamines afford the latter derivatives). Primary alkyl- and arylamines react with succinic dialdehyde to give a pyrrole derivative which is then developed with p-dimethylaminobenzaldehyde and they also yield fluorescent derivatives with fluoescamine. Primary and secondary alkylamines produce fluorescent 4-amino derivatives with 7-nitrobenzofurazan. Secondary alkylamines are selectively determined as N-substituted derivatives of 2-chloro-3-(2-aminoethyl)-5,6-dicyano-1,4-benzoquinone, or of 4-amino- or 4,5-diamino-1,2-benzoquinone. Only primary arylamines condense with glutaconic dialdehyde to yield a colored Schiff's base. Diazo coupling with p-nitrophenyldiazonium ion allows the estimation of all classes of arylamines. Tertiary alkylamines and quaternary ammoniums develop a color with *cis*-aconitic anhydride in the presence of acetic anhydride, whereas only tertiary alkylamines develop a fluorescence with a mixture of aconitic acid and acetic anhydride.

### INTRODUCTION

Numerous methods of colorimetric and fluorimetric determination of amines have been described in the literature. A definite reaction has often given rise to various procedures. A number of these methods are recommended which have been checked in the authors' laboratory and are considered reliable.

In the colorimetric determinations described, Beer's law is obeyed at least up to the absorbance value of 0.8. For the fluorimetric determination, a linear relationship is observed within the limits given. Smaller amounts of compounds can, however, sometimes be determined but then the linear relationship does not necessarily hold.

In all cases, the compounds given under Results, as well as the compounds which are given as reacting only weakly or not at all were the only compounds tested. Therefore, no reaction should be considered selective or limited to the compounds listed. It was not possible to test the hundreds of known amino compounds which are described in the literature; many of them interest only a few specialists, and some simple compounds were selected in order to illustrate the sensitivity of the procedures.

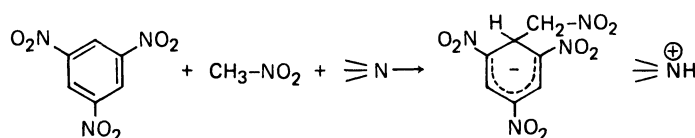
All the reagents used were of analytical grade, the solvents of reagent grade, and the water distilled. For all reagents in solution, the concentration is always expressed in v/w if the solute is a solid, and in v/v if it is a liquid. Unless otherwise stated, solutions of acids, bases and salts are always aqueous. For the sake of brevity, the limits within which a given temperature or a given time should be observed were not specified for each of the methods.

Temperature: "Heat at t° C," means the temperature should be maintained at t + 1° C. "Room temperature" means the temperature is within the range of 18-24° C. Time: "Let stand for x min" or "Heat for x min," means x min + 5%. "Let stand for x min and read" means start with reading after x min + 5%. The absorbance is always read against the reagent blank.

## A. ALIPHATIC AMINES AND QUATERNARY AMMONIUMS (Colorimetry)

Reaction with 1,3,5-trinitrobenzene and nitromethane (1)

Formation of a red Meisenheimer-type complex:

Chemicals

Ethanol, nitromethane, 1,3,5-trinitrobenzene, and silver oxide.

Reagent solution

An 0.20% solution of 1,3,5-trinitrobenzene in nitromethane. Store in the dark.

Methods

1. Free amines: To 0.20 ml of sample solution in ethanol, add 0.20 ml of reagent, mix, and let stand for 90 a at room temperature. Dilute with 3.0 ml of nitromethane and measure the absorbance at 565 nm.

2. Hydrochlorides and halides: To 0.20 ml of sample solution in ethanol, add approximately 5 mg silver oxide and 0.20 ml of reagent. Shake the tube for 2 min with an even oscillating motion and dilute with 3.0 ml of nitromethane. Decant, and measure absorbance immediately at 565 nm (the solid phase settles out easily; no filtration is necessary).

ResultsSample size ( $\mu\text{g}$ ) for  $A = 0.30$   
(1 cm - cell)

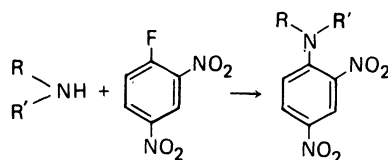
1. Free amines	
n-Butylamine	6.8
Di-n-butylamine	9.0
Triethylamine	7.5
2. Hydrochlorides and chlorides	
Acetylcholine	18.0
Benzyltrimethylammonium (iodide)	25.0
Dibenzylamine	25.0
Di-n-butylamine	12.5
Ethylamine	8.0
Methylamine	5.2
Promethazine	30.0
Triethylamine	10.5
Trimethylamine	13.0

Guanidine derivatives also react. The absorbance value 0.30 is obtained with 22  $\mu\text{g}$  of chlorguanide or 7  $\mu\text{g}$  of guanidine hydrochloride, which affords poor reproducibility. Various alkaloids can also be estimated. For instance,  $A = 0.30$  is given by 45  $\mu\text{g}$  of emetine hydrochloride or 50  $\mu\text{g}$  of morphine hydrochloride. Arylamines are almost unreactive.

## B. PRIMARY AND SECONDARY ALKYL- AND ARYLAMINES (colorimetry)

Reaction with 1-fluoro-2,4-dinitrobenzene (2)

Formation of a yellow-orange N-substituted 2,4-dinitroaniline. The excess of reagent is hydrolyzed to 2,4-dinitrophenol and the colored species is extracted into cyclohexane:

Chemicals

Cyclohexane, dioxane, ethanol, 0.10 M sodium bicarbonate, sodium hydroxide and 1-fluoro-2,4-dinitrobenzene.

Reagents

1. 1.20% solution of 1-fluoro-2,4-dinitrobenzene in ethanol.
2. 0.2 N solution of sodium hydroxide in a 6:4 mixture of dioxane and water.

Method

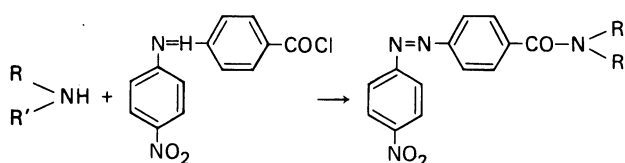
To 0.10 ml of neutralized aqueous solution of the amine, add 0.05 ml of Reagent 1 and 0.10 ml of reagent 0.10 M sodium bicarbonate. Heat at 60° C for 20 min, add 0.40 ml of Reagent 2 and keep this mixture for 1 h at 60° C. Dilute to 10.0 ml with water, extract the color into 10 ml of coclohexane and measure the absorbance at an appropriate wavelength.

Results

	$\lambda$ Max. (nm)	Sample size ( $\mu$ g) for A = 30 (1 cm - cell)
Aniline	340	19
Diethylamine	350	17
Di-n-propylamine	360	26
Ethylamine	330 - 390	12.5 - 30
N-Methylaniline	360	210
n-Propylamine	330 - 390	13.5 - 38

2. Reaction with P-nitrophenylazobenzoyl chloride (3)

Formation of an amide. The excess reagent is destroyed with hydrogen peroxide and the orange-red color developed in alkaline medium:

Chemicals

Dimethyl sulfoxide, dioxane, a 30% aqueous solution of hydrogen peroxide, a 40% solution of benzyltrimethylammonium hydroxide in methanol and p-nitrophenylazobenzoyl chloride.

Reagents

1. An 0.01% solution of p-nitrophenylazobenzoyl chloride in dioxane.
2. Dilute 2.0 ml of 30% aqueous solution of hydrogen peroxide to 100 ml with dimethyl sulfoxide.
3. Immediately before use, dilute 1.0 ml of 40% solution of benzyltrimethylammonium hydroxide in methanol to 100 ml with dimethyl sulfoxide.

Method

To 2.0 ml of sample solution in dioxane, add 1.0 ml of Reagent 1 and let stand at room temperature for 15 min in the dark. Add 0.50 ml of Reagent 2, 2.0 ml of Reagent 3, mix and measure the absorbance at an appropriate wavelength.

Results

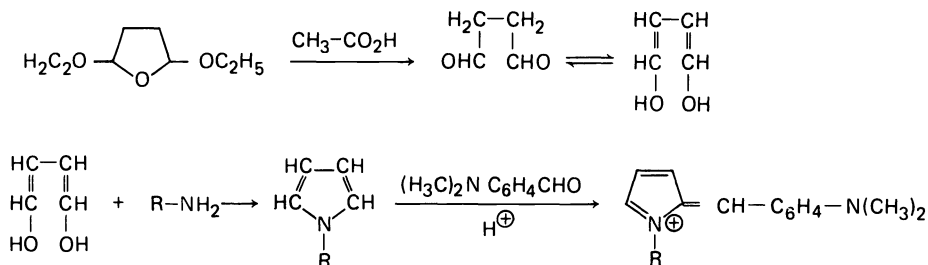
	$\lambda$ Max. (nm)	Sample size ( $\mu$ g) for A = 0.30 (1 cm - cell)
Aniline	525	54
Benzylamine	540	15.7
n-Butylamine	530	8.0
Dibenzylamine	530	57
Di-n-butylamine	530	42.5
Diethylamine	530	21.6
Di-n-propylamine	530	31.0
Ethylamine	535	6.2
N-Methylaniline	535	19.0
N-Propylamine	530	9.2

p-Aminobenzoic acid reacts, but weakly.

C. PRIMARY ALKYL- and  $\alpha$ -AMINO ACIDS (Colorimetry)

## Reaction with succinic dialdehyde and p-dimethylaminobenzaldehyde (4.5)

The succinic dialdehyde formed in the reaction medium by hydrolytic cleavage of 2,5-diethoxytetrahydrofuran reacts with the amine to give an N-substituted pyrrole which is developed with p-dimethylaminobenzaldehyde: pink-orange to pink-violet color:



## Chemicals

Ethanol, glacial acetic acid, concentrated hydrochloric acid, 2,5-diethoxytetrahydrofuran, and p-dimethylaminobenzaldehyde.

## Reagents

1. Dilute 1.0 ml of 2,5-diethoxytetrahydrofuran to 100 ml with glacial acetic acid.
2. A 2.0% solution of p-dimethylaminobenzaldehyde in a 1:19 mixture of concentrated hydrochloric acid and glacial acetic acid.

## Method

To 1.0 ml of sample solution in 50% ethanol, add 1.0 ml of Reagent 1 and heat at 80° C for T<sub>1</sub> min. Cool to room temperature in a water bath and add 3.0 ml of Reagent 2. Let stand at room temperature for T<sub>2</sub> min and measure the absorbance at an appropriate wavelength.

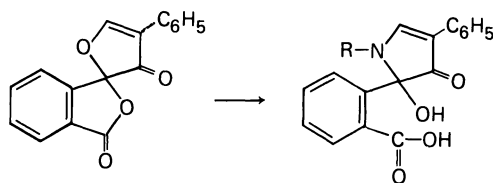
## Results

	T <sub>1</sub> (min)	T <sub>2</sub> (min)	$\lambda$ Max. (nm)	Sample size ( $\mu$ g) for A - 0.30 (1 cm - cell)
Aniline	2	15	558	2.1
Benzylamine	2	5	558	2.8
n-Butylamine	20	2	558	1.9
n-Propylamine	20	2	558	1.5
Sulfanilamide	2	45	558	5.0
p-Toluidine	2	5	558	2.1
Arginine (dihydrate)	10	30	515	7.4
Cysteine	10	30	545	7.4
Glycine	10	5	558	1.8
Phenylalanine	10	20	558	5.7
Threonine	10	30	507	3.3

D. PRIMARY ALKYL- AND ARYLAMINES, AND  $\alpha$ -AMINO ACIDS (Fluorimetry)

## Reaction with fluorescamine

It should be mentioned how fluorescamine, or 4-phenylspiro[furan-2 (3H) 1'-phthalan]-3,3'-dione was introduced as reagent. It was shown that, upon reaction with ninhydrin and phenylacetaldehyde, primary alkylamines and  $\alpha$ -amino acids developed a green fluorescence which allowed very sensitive determinations (6). The general structure of the fluorescent species was then established from the derivative given by ethylamine (7) and led to the synthesis of fluorescamine, which condensed directly with primary alkyl- and arylamines, and  $\alpha$ -amino acids, to yield the fluorescent species (8). The reaction has given rise to various procedures and numerous applications. The method herein proposed was developed in our laboratory:



Chemicals

Acetone, carbon dioxide-free 0.20 M sodium hydroxide, boric acid, potassium chloride, and fluorecamine.

Buffers

To 50.0 ml of an 0.20 M solution of both boric acid (12.368 g in 1 liter) and potassium chloride (14.90 g in 1 liter), add 10.25 ml (pH 8.5) or 21.30 ml (pH 9.0) of 0.20 M sodium hydroxide in carbon dioxide-free solutions and dilute to 200 ml with carbon dioxide-free distilled water.

Reagent

An 0.02% solution of fluorecamine in acetone. This reagent is stable for one day.

Method

To 0.50 ml of a neutralized aqueous solution of the sample, add 0.50 ml of buffer (pH 8.5 for alkyl- and arylamines, pH 9.0 for -amino acids) and mix. While shaking the tube, add 0.50 ml of reagent, dilute with 3.0 ml of water, and measure the absorbance.

Results

	Excitation $\lambda$ (nm)	Emission $\lambda$ (nm)	Determination limits ( $\mu\text{g}$ )
p-Aminobenzoic acid	410	500	0.12 - 0.60
Aniline	400	500	0.10 - 0.50
o-Anisidine	400	505	2.0 - 10.0
Benzylamine	395	485	0.10 - 0.50
n-Butylamine	395	485	0.20 - 1.0
Cyclohexylamine	395	485	0.40 - 2.0
Ethylamine	395	485	0.20 - 1.0
Methylamine (hydrochloride)	395	485	0.40 - 2.0
$\beta$ -Naphthylamine	410	520	1.0 - 5.0
n-Propylamine	395	485	0.15 - 0.75
Sulfanilamide	400	490	0.40 - 2.0
m-Toluidine	410	510	0.20 - 1.0
o-Toluidine	395	485	0.40 - 2.0
p-Toluidine	410	515	1.0 - 5.0

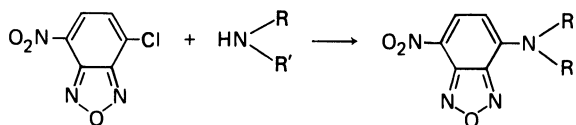
For all the  $\alpha$ -amino acids tested, the excitation  $\lambda$  was 395 nm; the emission  $\lambda$  was 485 nm.

$\alpha$ -Alanine	0.40 - 2.0
Arginine	0.40 - 2.0
Cystine (dihydrochloride)	0.30 - 1.5
Glutamine	0.30 - 1.0
Glycine	0.20 - 1.0
Histidine (hydrochloride)	0.40 - 2.0
Leucine	0.20 - 1.0
Lysine (hydrochloride)	0.40 - 2.0
Serine	0.20 - 1.0
Threonine	0.20 - 1.0

## E. PRIMARY AND SECONDARY ALKYLAMINES (Fluorimetry)

Reaction with 4-chloro-7-nitrobenzofuran (9)

Formation of a 4-mono- or dialkylamino-7-nitrobenzofuran, which exhibits a yellow fluorescence:

Chemicals

Ethanol and 4-chloro-7-nitrobenzofuran (7-chloro-4-nitrobenzoxadiazole).

Reagent

An 0.05% solution of 4-chloro-7-nitrobenzofuran in ethanol. Store in the dark.

Method

To 1.0 ml of sample solution in ethanol, add 0.75 ml of reagent and heat at 60°C for 30 min, exposed to daylight. Cool to room temperature and let stand for 3 min and dilute to 4.0 ml with ethanol. Measure the absorbance at excitation of 436 nm; emission 535 nm.

Results

	Determination limits ( $\mu\text{g}$ )
Benzylamine	0.25 - 1.0
n-Butylamine	0.25 - 1.0
Di-n-butylamine	2.0 - 10.0
Diethylamine	1.0 - 5.0
Di-n-propylamine	3.0 - 12.0
n-Propylamine	0.25 - 1.0

Tertiary alkylamines do not react. The reaction is very weakly positive with arylamines, phenols, and mercaptans.  $\alpha$ -Amino acids develop a fluorescence, but the linear relationship is not observed.

## F. PRIMARY ALKYLAMINES (Colorimetry)

Reaction with 1-fluoro-2,4-dinitrobenzene (10)

Formation of an N-alkyl 2,4-dinitroaniline. Under the given conditions, only primary alkylamines can be estimated (compare with method B, 1): violet color.

Chemicals

Dimethylformamide, nitromethane, a 40% solution of benzyltrimethylammonium hydroxide in methanol and 1-fluoro-2,4-dinitrobenzene.

Reagents

1. An 0.025% solution of 1-fluoro-2,4-dinitrobenzene in dimethylformamide.
2. Just before use, dilute 1.0 ml of 40% solution of benzyltrimethylammonium hydroxide in methanol to 100 ml with dimethylformamide.

Method

To 1.0 ml of sample solution in dimethylformamide, add 0.50 ml of Reagent 1 and let stand for 10 min at room temperature. Add 4.50 ml of Reagent 2 and 0.10 ml of nitromethane, and let stand for 10 min at room temperature. Measure the absorbance at 570 nm.

Results

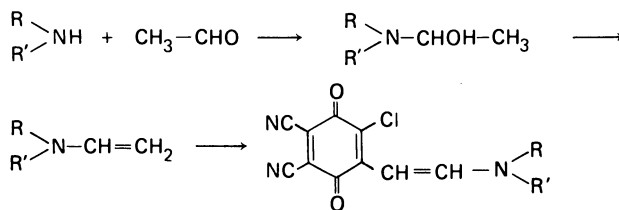
	Sample size ( $\mu\text{g}$ ) for A = 0.30 (1 cm - cell)
Benzylamine	15.2
Cyclohexylamine	17.0
Ethylamine	5.7
Monoethanolamine	7.8
n-Propylamine	7.6

Secondary alkylamines react weakly. The reaction is negative with arylamines and tertiary alkylamines.

## G. SECONDARY ALKYLAMINES (Colorimetry)

1. Reaction with acetaldehyde and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (11)

The mechanism of the reaction is probably similar to that proposed for the reaction of primary and secondary alkylamines with acetaldehyde and chloranil (12) violet color.



Chemicals

Dioxane acetaldehyde, and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

Reagents

1. An 0.055% solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in dioxane.
2. A 5.0% solution of acetaldehyde in dioxane.

Method

To 1.0 ml of sample solution in dioxane, add 0.10 ml of Reagent 1 and 0.10 ml of Reagent 2, and heat at 70°C for 3 min. Cool for 1 min in ice water, add 2.5 ml of dioxane, and measure the absorbance at 560 nm.

Results

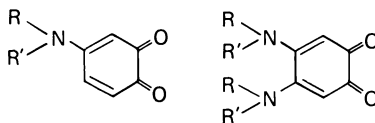
Sample size ( $\mu\text{g}$ ) for  $A = 0.30$   
(1 cm - cell)

Dibenzylamine	33
Di-n-butylamine	14
Di-n-propylamine	11
N-Methylbenzylamine	13

Positive reaction is also given by piperidine ( $A = 0.30$  for 18  $\mu\text{g}$ ) and morpholine ( $A = 30$  for 11  $\mu\text{g}$ ). Sensitive results are also obtained with pyrrolidine, but Beer's law is not followed. Arylamines and primary and tertiary alkylamines practically do not react, but primary alkyl-amines interfere with the determination.

## 2. Reaction with pyrocatechol and silver oxide (13)

Formation of a 4-amino- or a 4,5-diamino-1,2 benzoquinone; violet color. (14)

Chemicals

Acetone, silver oxide, and pyrocatechol.

Reagent

An 0.10% solution of pyrocatechol in acetone.

Methods

1. Free amines: To 0.50 ml of sample solution in acetone, add 1.0 ml of reagent and about 2 mg of silver oxide. Shake for a few seconds and let stand for 10 min. Add 2.0 ml of acetone, decant (filtration is not necessary), and measure the absorbance at 510 nm.
2. Hydrochlorides: To 0.50 ml of aqueous solution of the amine hydrochloride, add 1.0 ml of reagent, 2.0 ml of acetone, and about 2 mg of silver oxide. Shake for a few seconds and let stand for 1 h. Decant (filtration is not necessary) and measure the absorbance at 510 nm.

Results

Samples size ( $\mu\text{g}$ ) for  $A = 0.30$   
(1 cm - cell)

1. Free amines	
Di-n-butylamine	45
Diethanolamine	60
Diethylamine	24
2. Hydrochlorides	
Adrenaline	70
Diobenzylamine	75
Di-n-butylamine	53
Diethanolamine	95
Diethylamine	28
Dimethylamine	30
Ephedrine	90

Unline appreciable amounts of primary amines, tertiary amines do not interfere.



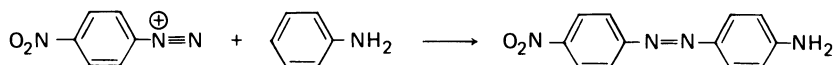


Since the fluorescence is very sensitive to the exciting light, only one reading per tube is possible. Primary amines do not react; secondary amines give a very transient fluorescence.

### I. ARYLAMINES (Colorimetry)

#### Reaction with p-nitrophenyldiazonium fluoborate (17,18)

All classes of arylamines couple with p-nitrophenyldiazonium ion in alkaline medium to produce products which exhibit a variety of colors:



#### Chemicals

Dimethylformamide, a 10% aqueous solution of tetramethylammonium hydroxide, and p-nitrophenyldiazonium fluoborate.

#### Reagent

An 0.50% aqueous solution of p-nitrophenyldiazonium fluoborate. Prepare immediately before use and filter.

#### Method

To 1.0 ml of aqueous solution of the amine, add 0.10 ml of reagent, 0.20 ml of 10% aqueous solution of tetramethylammonium hydroxide, and 10.0 ml of dimethylformamide. Mix, and measure the absorbance immediately.

#### Results

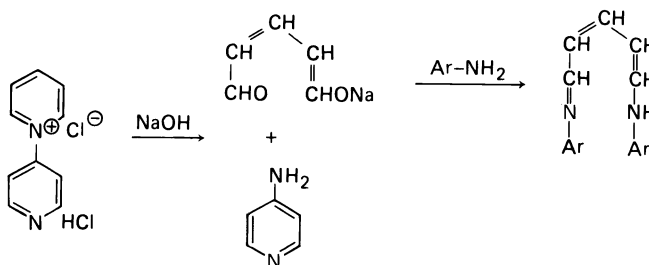
	$\lambda$ Max. (nm)	Sample size ( $\mu\text{g}$ ) for A = 0.30 (1 cm - cell)
p-Aminobenzoic acid	520	20
Aniline	530	6.0
N,N-Dimethylaniline	500	29
N-Methylaniline	425	80
Sulfanilamide*	530	250

\*Let stand for 15 min before reading.

### J. PRIMARY ARYLAMINES (Colorimetry)

#### Reaction with 4-pyridylpyridinium dichloride (19,20)

4-Pyridylpyridinium dichloride is decomposed in alkaline medium, giving rise to the enol form of gluconic aldehyde which reacts with the amine to give a condensate of yellow color Schiff's base:



The 4-aminopyridine formed does not behave like an arylamine and, therefore, does not interfere.

#### Chemicals

2 M sodium hydroxide, 2 M hydrochloric acid, and 4-pyridylpyridinium dichloride.

#### Reagent

A 1.0% aqueous solution 4-pyridylpyridinium dichloride.

#### Method

To 1.0 ml of aqueous solution of the amine, add 1.0 ml of 2 M sodium hydroxide, 1.0 ml of reagent, and 2.0 ml of 2 M hydrochloric acid. Let stand for 5 min and measure absorbance.

Results

	$\lambda$ Max. (nm)	Sample size ( $\mu$ g) for A = 0.30 (1 cm - cell)
p-Aminobenzoic acid	470	4.2
Aniline	455	5.4
p-Anisidine	455	5.6
Procaine (hydrochloride)	470	7.6
Sulfanilamide	470	6.5
p-Toluidine	455	5.4

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