Oriental Journal of Chemistry Vol. **26**(4), 1419-1424 (2010)

Spectrophotometric determination of trace amount of some aromatic amines in pharmaceutical formulations, wastewater and biological fluids

K.C. SRINIVASA MURTHY

Department of Chemistry, R.L. Jalappa Institute of Technology,
Kodigehalli, Doddaballapur-561 203, India.

(Received: August 09, 2010; Accepted: September 16, 2010)

ABSTRACT

A simple, accurate, sensitive and rapid method for the determination of some aromatic amines in either the pure form or in pharmaceutical formulation or in biological fluids or in waste water sample is proposed. The method is based on the formation of colored compounds with sodium 1,2-naphthoquinone-4-sulphonate and cetyltrimethyl ammonium bromide in alkaline medium. Beer's law of colored species is obeyed in the range of concentration of 0.5 – 24 µg ml^{-1}. The molar absorptivity was found to be in the range of $3.7504x10^3$ – $1.1180x10^4$ l mol^{-1} cm^{-1}. Results obtained by using the proposed method for various pharmaceutical preparations compare favorably with those obtained by the official method.

Key words: Aromatic amines, Pharmaceutical formulations, Waste water and Biological fluids.

INTRODUCTION

The determination of aromatic amines like aniline is very important from the point view of environmental protection, chemical processes and toxicology. These compounds are highly toxic and should be determined, particularly in the effluents from the industrial plants where aromatic amines are used. The majority of published spectrophotometric methods for the determination of aromatic amines were involved diazotization and reaction with a suitable coupling agent to produce intensely colored azo dyes[1-3]. Those methods leave much to be desired in the way of accuracy and reproducibility for the determination in micrograms.

Sanghavi et al.[4] and Josef Jenik[5] reported a colorimetric methods. Former estimated hydrolysed product 4-aminophenol of paracetamol (PRL) with 1,2-naphthoquinon-4-sulphonate (NQS) in acid medium to form a Schiff base having an absorption maximum at 480 nm. Latter estimated organic amino compounds with same reagent at the pH ~ 5 (λ max.: 460 nm). However, in our laboratory, above reactions were carried out and it gives a yellow color instead of a red or reddish yellow color, and a red color in alkaline medium.

In the present communication we extend our reported method[6] for the determination of isoniazed to some aromatic amines, based on the formation of colored products with NQS and, and cetyltrimethyl ammonium bromide (CTA) in alkaline medium. The method offers the advantages of sensitivity, simplicity and rapidity without need for extraction.

MATERIAL AND METHODS

Apparatus

A Shimadzu Double beam spectrophotometer UV-150-02 with 1.0 cm matched cells was used for spectral measurements.

Reagents

Stock solutions of aromatic amines viz., aniline, AL; p-bromoaniline, PBA; p-chloroaniline, PCA; p-toluidine, PTD (BDH Corp., India) were prepared by dissolving 100 mg of each substance

in separate 100 ml calibrated flasks in 5 ml of ethanol and completing to volume with distilled water.

Accurately weighed (100 mg) of paracetamol (BDH Corp., India) or phenacetin, PHN (BDH Corp., India) was transferred to a 100 ml round bottom flask containing 15 ml of 20 % hydrochloric acid, and refluxed for 30 min. The solution was cooled and the condenser washed with water. The solution with the washings was transferred to a 100 ml calibrated flask and diluted to volume with water. The resultant hydrolyzed solution contains p-aminophenol[7] (PAP) or p-phenetidine[8] (PHD) and standardized by a reported method[9]. Working standard aromatic amine solutions of lower concentration (50 µg / ml) were prepared by appropriate dilution of the stock solutions with water. A 0.02 % aqueous solution of NQS (BDH Corp., India) was freshly prepared and protected from sunlight. A 1 % aqueous solution of CTA (Hopkins and Williams, UK), 0.01 M, and 2 % sodium hydroxide solution and 2 % sodium carbonate solution were used.

All reagents used were of analytical reagent grade when not mentioned and deionized water was used to prepare all solutions and in all experiments.

Procedure

Aliquots of standard solutions of AL (12.5-275 µg ml^{-1}), PBA (25-435 µg ml^{-1}), PCA (35-400 mg ml^{-1}), PTD (25-300 µg ml^{-1}), PAP (25-500 µg ml^{-1}) or PHD (50-600 µg ml^{-1}) were transferred to a 25 ml calibrated flask, to which 6.0 ml of 0.02 % NQS, 1 ml of 1% CTA and 1.0 ml of 0.01M (for PAP: 2 ml of 2 %) NaOH; for PHD: 3 ml of 2 % Na_2CO_3 were added. Complete to volume with water and mixed well. The absorbance at λmax was measured against the corresponding reagent blank and calibration graphs were constructed.

Pharmaceutical formulations and laboratory made samples

As PHN formulations were not available in the Indian market, we prepared our own according to the literature method[10, 11]. Twenty tablets (commercial tablets or laboratory made tablets containing talc, starch, glucose and magnesium stearate) were powdered and weighed. An amount of equivalent to 50 mg (for syrup and injection form an appropriate volume of the samples) of PRL and PHN was taken and subjected to hydrolysis using 15 ml 20 % hydrochloric acid. The filtrate was made up to 100 ml and an aliquot of this solution was containing PAP and PHD was treated as described above for the determination of PAP or PHD.

RESULTS AND DISCUSSION

Spectral characteristics

Aromatic amines (AL, PBA, PCA, PTD, PAP and PHD) reacts with NQS in the presence of NaOH (for PHD, Na_2CO_3) in an aqueous medium to form a reddish-yellow to reddish violet colored anionic product with absorption maxima at 455 nm to 530 nm. When CTA was added to the anionic product, an intense orange red, red or violet colored product was obtained with a strong bathochromic shift of 20 to 40 nm (λ Max. 480 nm – 570 nm). This displacement is due to the substitution of CTA with anionic product. Hence, these wavelengths were used for all subsequent measurements.

Optimum reagent concentration and order of addition

It was found that a 0.02 % solution of NQS in the range 4 – 8 ml and a 1% of CTA in the range 0.5 – 2.0 ml were necessary to achieve maximum color intensity. Hence, 6 ml of NQS and 1 ml of CTA solution were selected. There was no appreciable change in the absorbance or color of the product if the order of addition of reactions was varied. The details of optical characteristics are summarized in Table 1.

Choice and effect of alkali

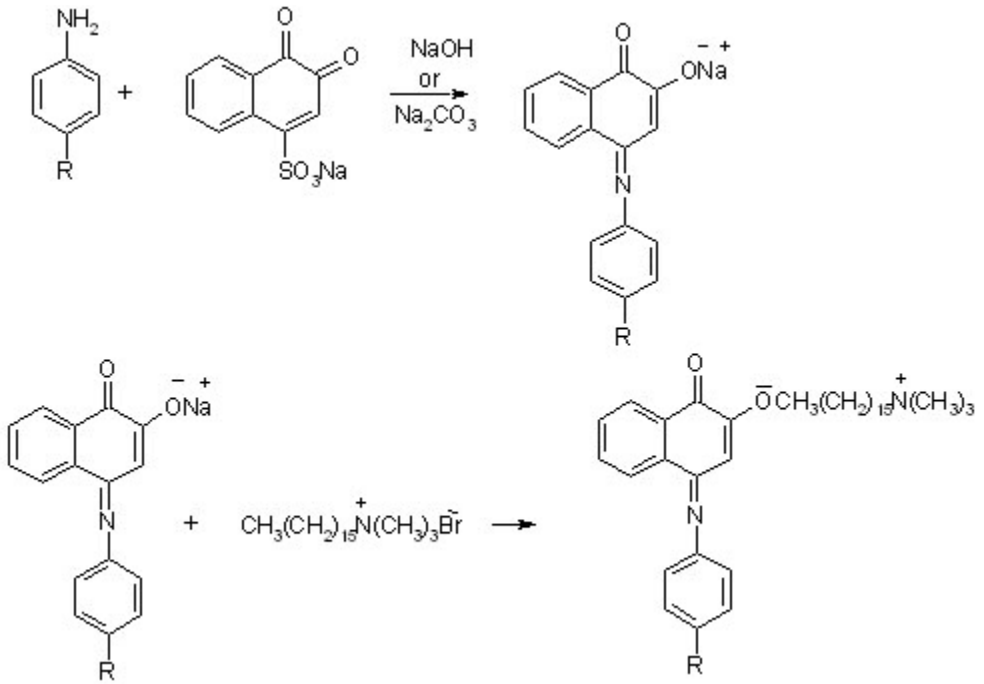
Choice of alkali is important for the investigation of the aromatic amines. Solutions of AL, PBA, PCA or PTD were slightly basic, because of its amine group. When adding NQS to above cited amines, within a few seconds red colored solid was separated. When adding 1.0 ml of 0.01 M NaOH solution, red colored solid is dissolved. For the above cited amines, various volume of 0.01M NaOH was tried and good results were obtained between 0.5 – 1.5 ml for the stable colored product. However, solution of PAP and PHD (hydrolyzed product of PRL and PHN) are in acidic medium. Consequently,

it required higher concentration of alkali solution. For PAP, various volumes of 2.0 % NaOH were tried and good results are obtained between 1.0 – 4.0 ml for the stable violet product. The red colored product of PHD was unstable in NaOH, while it is stable in 2.0 % Na_2CO_3 and the best results were obtained with between 1.0 to 6.0 ml of Na_2CO_3. The color intensity of the product is decreased when below the lower volume of above mentioned base solutions. Therefore, 2.0 ml of 2.0 % NaOH and 3.0 ml 2.0 % Na_2CO_3 were chosen for the investigation.

Reaction Sequence

A red colored solid product was separated when NQS is added to AL and it was recrystallized using ethanol. Aliqout of standard recrystallized product were analyzed using an LCMS (Thermo electron corporation, Model: Finnigan LCQ Advantage Max with Finnigan surreyor PDA plus detector) with 2.1 mm x 150 mm, 5µm Zorbax C 18 column at 30 °C (243 nm). A binary gradient at a flow rate of 0.6 mL / min used; mobile phase A contained 0.1 % trifluoroacetic acid in water and mobile phase B was 100 % acetonitrile. The LCMS spectrum indicates that analyte was confirmed based on the retention time (8.14 min), purity of analyte is 99.0 % and mass of the analyte is 249.3 (theoretically calculated molecular mass is 248).

Based on the Job's method of continuous variation it was found that selected analyte interacted with NQS in the ratio of 1:1. This lends support to the assumption that a paraquinoid imide condensation product is formed[6, 12-14]. Accordingly, the following sequence of reaction could be suggested. Under experimental conditions, light yellow alkaline solution of the orthoquinoidal NQS reacts with aromatic amines containing two removable hydrogen atoms attached to nitrogen atom, to yield an anionic reddish yellow or reddish violet colored paraquinoid imide condensation product. When CTA is added to the anionic condensation product, an intense orange, red or violet colored product is formed. A reaction mechanism based on the above reaction is shown in Fig. 1.



Where, R: H (Aniline); Br (p-Bromoaniline); Cl (p-Chloroaniline); CH_3 (p-toluidine); OH (p- aminophenol); OC_2H_5 (p-phenitidine)

Fig. 1: Reaction mechanism

Stability of the colored product

The products resulting from the suggested method was studied at different temperatures. It was found that the absorbance values remain constant in the temperature range 5 – 80 °C. Above 80 °C, the absorbance decreases, indicating that dissociation. Hence room temperature was recommended for the proposed method. These colored products were stable for more than 4 days and the results were reproducible.

Interference

The effects of the presence of excipients associated with the drug on the determination of PRL, in pure and dosage forms were investigated using the proposed method. These results indicate that dextrose, talc, starch, lactose, glucose, magnesium stearate, stearic acid, sodium alginate and gelatin do not interfere in pharmaceutical formulations in amount far in excess of their normal occurrence.

The effects of various substances that often accompany PRL in pharmaceutical preparations were studied. Of the drugs commonly contained in formulations of PRL, indomethacin, promethazine, chlorpheniramine maleate, phenylephrine HCl and ascorbic acid interfered, while analgin, caffeine, ibuprofen, diclofenac sodium, chlorzoxaone, benzyl alcohol did not interfere in the determination of PRL by the proposed methods (Table 2).

Application

The applicability of method to assay of pharmaceutical preparations was examined. The Table 2 shows, the results obtained on the assay of PRL preparations singly and in various combinations. The results obtained compare favorably with the results obtained by the official method[15].

The validity of the proposed method was examined for the determination of aniline in waste water and biological samples. Waste water

Table 1: Parameters for the spectrophotometric determination of aromatic amines

Optical character	AL	PBA	PCA	PTD	PAP	PHD
Color	Orange red	Orange red	Orange red	Orange red	Violet	Red
λ_{max} (nm)	490	490	480	480	570	500
Beer's law ($\mu g\ ml^{-1}$)	0.5 – 11.0	1.0 -17.4	1.4 – 16.0	1.0 – 12.0	1.0 – 20.0	2.0 – 24.0
Molar absorptivity ($l\ mol^{-1}\ cm^{-1}$)	5.8860 $x10^3$	5.8904 $x10^3$	3.7504 $x10^3$	5.8304 $x10^3$	1.1180 $x10^4$	4.8190 $x10^3$
Specific absorptivity ($\mu l\ g^{-1}\ cm^{-1}$)	0.0632	0.0342	0.0294	0.0544	0.0740	0.0269
Sandell's Sensitivity ($mg\ cm^{-2}$)	15.8 x 10^{-3}	29.2 x 10^{-3}	34.0 x 10^{-3}	18.4 x 10^{-3}	14.0 x 10^{-3}	37.17 x 10^{-3}
Optical photometric range ($mg\ ml^{-1}$)	1.1– 9.6	1.2 -16.5	2.5 – 15.5	1.9 – 11.7	1.2 – 19.4	4.0 -23.4
Regression equation (y*):Slope (a)	0.0637	0.0331	0.0313	0.0544	0.0718	0.0257
Intercept (b)	-0.0043	0.0087	-0.0073	-0.0020	0.0031	0.0089
Correlation coefficient (r)	1.0000	0.9867	0.9999	0.9973	1.0040	1.0000
% RSD #	1.40	1.20	1.00	1.60	1.44	2.70
% Range of Error @	± 0.58	± 0.75	± 0.69	± 0.45	± 0.55	± 0.78

* $y = ax + b$, where 'x' is the concentration ($\mu g\ ml^{-1}$) of aromatic amines

\# Calculated from five determinations (n=5)

@ Average of three determinations

Table 2: Determination of Paracetamol and Phenacetin by the proposed method

Products	Composition (mg)	% Recovery # ± % RSD @ Proposed method	Official method[15]
Dolopar *			
Paracetamol	250	99.6 ± 0.56	99.4 ± 1.10
Analgin	250		
Caffeine	250		
Calpol *			
Paracetamol	500	100.0 ± 1.12	100.1 ± 0.65
Pacimol *			
Paracetamol	500	100.2 ± 0.78	99.9 ± 0.81
Pyrigesic *			
Paracetamol	500	99.9 ± 0.32	99.9 ± 0.53
Ibugesic Plus *			
Ibuprofen	200		
Paracetamol	325	1001.5 ± 0.59	101.5 ± 0.73
Diclogesic *			
Diclofenac sodium	50		
Paracetamol	500	100.7 ± 0.83	100.4 ± 0.49
Flamar-P *			
Chlorzoxaone	250	100.0 ± 0.67	99.9 ± 0.81
Paracetamol	300		
Dolo (syrup) *		100.5 ± 0.77	100.3 ± 0.99
Paracetamol	500		
Crocin Drops *		100.9 ± 1.13	100.2 ± 0.54
Paracetamol	150		
H-Mol-75 *		99.8 ± 0.99	99.8 ± 1.21
Paracetamol	75		
Benzyl alcohol			
Phenacetin (laboratory made**)	200	100.1 ± 0.39	——-

* Trade name of the drugs: # Average of 5 determination, assay as a % of label claim.
@ Relative standard deviations (n=5): ** not available in Indian market.

Table 3: Determination of aniline in spiked waste water samples

Concentration of aniline added (mg ml^{-1})	Absorbance	Aniline found* (µg ml^{-1})	% Recovery
4	0.252	3.88	97.0
6	0.390	6.06	101.0
8	0.515	8.02	100.3
10	0.649	10.12	101.2

* Mean of five repeated determination

Table 4: Determination of aniline in spiked urine samples

Sample	Aniline added ($\mu g\ ml^{-1}$)	Aniline found *	% of Recovery
S_1	6	6.0	100.0
S_2	8	7.8	97.5
S_3	10	9.8	98.0

* Mean of five repetitive determinations

Samples were collected from the source of industrial effluents and from the near by Kukkarahalli lake at Mysore and suspended particles were removed by filtration. The clear waste water samples and human urine samples are analyzed for aniline using the above recommend procedure and found to be free from aniline. So that waste water sample and urine sample were spiked with known amount of aniline and analyzed as above. The results were summarized in Table 3 and Table 4. The data show that the recovery of aniline is quantitative. Absorbance of the product (Table 3) has a linear relationship at the level of investigated to concentration of aniline in the original sample. Hence, the proposed method can be satisfactorily applied to hygiene work.

CONCLUSION

The proposed method has the advantages of rapidity, simplicity and sensitivity. The assay method involve less stringent control of experimental parameters, such as time of analysis, the stability of the colored species, and the concentration of the reagent. The utility of the proposed method for PRL in dosage forms and AL in waste water and urine sample has been demonstrated. The lower value of relative standard deviation reflects reproducibility of the proposed method. Thus it can be concluded that this method could be considered for the assay of both bulk and formulations and successfully applied to both industrial and hygiene work.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to Management and Principal of R.L.Jalappa Institute of Technology, Doddaballapur for their constant encouragement.

REFERENCES

1. Agarwal S.P and Walash M.I., *Indian J. Pharm. Sci.,* **36**, 47 (1974)
2. George Norwitz and Peter N. Keliher, *Anal. Chem.*, **53,** 1238 and 56 (1981)
3. Esteve Romero J. S. and Alvarez Rodriguez L. *Analyst,* **119,** 1381 (1994).
4. Sanghavi N. M. and Pai M.G., *East. Pharm.,* **17**, 53 (1974)
5. Josef Jenik, *Chemickotechnol Pardubice*, **40,** 133 (1979**)**
6. Srinivasa murthy K.C., Nagaraja P. and Yathirajan H.S., *Talanta*, **43**, 1075 (1996**)** and references cited therein.
7. Connors K., Amidon G. and Kennon L., Chemical Stability of Pharmaceuticals, Wiley-Interscience Publications, NY, 123 (1979)
8. Higuchi T. and Brochmann-Hanssen E., Pharmaceutical Analysis, Interscience Publishers, New York, NY, .551 (1961)
9. Koka I.P., Farmatsiya, **33,** 37 (1984)
10. Liberman H.A. and Lachman L. (Eds), Pharmaceutical Dosage Forms: Tablets, **Vol. I** Dekker, New York, (1980)
11. Lachman L., Liberman H.A. and Kanig J.L. (eds), The theory and Practice of Industrial Pharmacy, 2nd Edn. Henry Kimpton, London (1976)
12. Saurina J., Hemandez-Cassou S. and Tauler R., *Anal. Chem.* **67,** 3722 (1995)
13. Ehrlich P. and Herter C.A, *Z. Physiol. Chem.*, **41**, 329 (1904)
14. Roth H.J., Eger K. and Troschutz R., Phrmazeutische Chemie II, Arzneistoff analyse, Georg Thieme Verlag, *Stuttgart*, 279, 280 (1981)
15. British Pharmacopoeia, Office of the British Pharmacopeia Commission, London, 483 (1993).