RESEARCH ARTICLE

ScienceAsia 28 (2002) : **359-364**

Spectrophotometric Determination of Astemizole and Loratadine Based on Charge-Transfer Complex Formation with Chloranilic Acid

K Basavaiah* and V S Charan

Department of Chemistry, University of Mysore, Manasagangothri, Mysore – 570 006, Karnataka, India.

Corresponding author, E-mail: basavaiahk@yahoo.com.in

Received 10 Oct 2001
Accepted 13 May 2002

ABSTRACT A simple and sensitive spectrophotometric method for the assay of two antihistamines, astemizole and loratadine, is described. The determination was based on the formation of charge-transfer complexes between chloranilic acid as a p - acceptor and the studied drugs as n-donors in acetonitrile solvent. The spectra, various experimental parameters, composition and formation constant of the complexes were studied. The formation constant (K) values of CT complex with astemizole were $0.9x10^3$ and $1.06x10^3$ calculated from Benesi-Hildebrand plot and from Turner and Anderson plot, respectively, whereas the corresponding values for loratadine were $4.50x10^3$ and $4.19x10^3$, respectively. The charge-transfer complexes formed were found to absorb at 520 nm. Beer's law was obeyed in the concentration range 10 - 120 and 15 - 210 μg ml^{-1} for astemizole and loratadine respectively. The apparent molar absorptivity and Sandell sensitivity values were $1.78x10^3$ and $0.96x10^3$ l mol^{-1} cm^{-1} and 257.62 and 396.61 ng cm^{-2}, for astemizole and loratadine, respectively. The limits of detection and quantification for astemizole were 1.05 and 3.51 μg ml^{-1}, respectively; the corresponding values for loratadine were 2.21 and 7.39 μg ml^{-1} respectively. The method was applied to the determination of the studied drugs in several of their commercial preparations and the results were statistically validated. Excipients used as additives in commercial formulations did not interfere in the proposed procedure.

KEYWORDS: ?????.

INTRODUCTION

Astemizole (AST) is chemically 1-(4-fluorobenzyl) -2- {[1-(4-methoxyphenyl) – 4 – piperidyl] – amino} – benzimidazole. It is a long-acting, selective histamine H_1 receptor antagonist. It is a second-generation antihistamine in that it does not readily cross the blood-brain barrier. Astemizole is used in the treatment of both seasonal and perennial allergic rhenitis, allergic conjunctivitis, chronic urticaria and other chronic allergies. Quantitation of astemizole has been carried out by high-performance liquid chromatography with uv detection.[1,2] Several spectrophotometric procedures based on redox reaction involving N-bromosuccinimide (NBS) celestine blue[3], ion-association complex formation using acid dyes[4] and complex formation utilising N-bromosuccinimide[5] in alkaline medium have been proposed for the assay of the drug in bulk drug as well as in dosage forms. The reliability of the NBS – celestine blue method[3] depends on the purity of the dye which is never available in pure state whereas the acidic dye method[4] involves tedious extraction steps in addition to careful control of pH. The NBS method[5] makes use of flow injection assembly, which is not always readily available.

Loratadine (LRT) is also a antihistamine H_1 receptor antagonist whose action is characterized by quick onset and long duration. It is widely used to treat seasonal and perennial allergic rhinitis, allergic dermatitis and urticaria and ocular allergy. Techniques such as gas liquid chromatography[6], high-performance liquid chromotography[7] and GC– mass spectrometry[8] have been used to determine loratadine in body fluids. The literature survey reveals that there is only visible spectrophotometric method based on ion-association complex formation with bromophenol blue[9] in acidic buffer medium for the assay of loratadine in dosage forms. Squella et al[10] have described a polarographic method which requires nitration of the compound before subjecting to electrolysis. Loratadine in tablets has also been quantitated by uv-spectrophotometry[11] but the method is non-specific.

The molecular interactions between electron donors and acceptors are generally associated with

the formation of intensely coloured charge-transfer complexes[12] which absorb radiation in the visible region. The photometric methods based on these interactions are usually simple and convenient[13] because of the rapid formation of the complexes. Both the drugs are good n-electron donors (Fig 1) and will form charge-transfer complexes with organic p-acid, chloranilic acid. These types of complexes are used in the determination of the components of the complexes.[14,15] Application of chloranilic acid (CAA) for the determination of some medicinal compounds containing basic nitrogen atom has recently been described.[16-21]

The present work describes the utility of CAA reagent for the spectrophotometric determination of AST and LRT in pure form as well as in their dosage forms. In addition, the composition and stability constant of the charge-transfer complexes formed between the drugs and the reagent were also established.

Experimental

Apparatus

A systronics model 106 digital spectrophotometer (340-960 nm) with 1 cm glass cells were used for absorbance measurements.

Reagents

Pharmaceutical grade AST (Torrent Pharmaceuticals Ltd,) and LRT (Morepen Labs Ltd,) were used as working standards. CAA (sd Fine Chem India Ltd,) solution, 0.1%, was freshly prepared in acetonitrile. All other reagents and solvents used were of analytical grade.

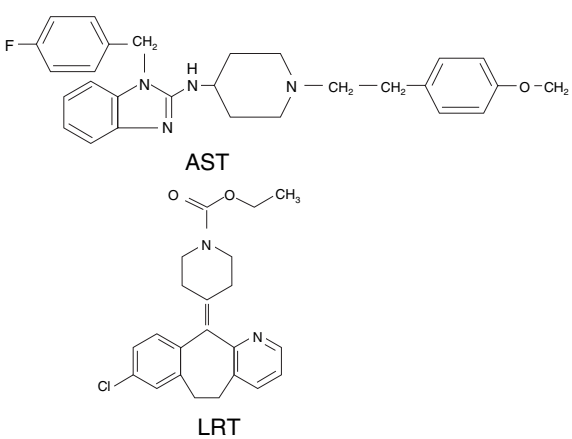

Fig 1. Structures of the antihistamines studied.

Standard Solutions

An accurately weighed amount of drug AST or LRT are equivalent to 100 mg of the base was dissolved in about 20 ml chloroform and made upto volume into a 100 ml volumetric flask with chloroform to provide a 1 mg ml^{-1} stock standard solution of the base. This was diluted to get a working concentration of 200 μg ml^{-1} and 300 μg ml^{-1} for AST and LRT, respectively.

Procedures

Calibration Graphs

Serial volumes of 0.25 to 3.00 ml (AST) or 0.25 to 3.5 ml (LRT) were transferred into 5 ml volumetric flasks and the volume was brought to 3.0 or 3.5 ml by adding requisite volumes of chloroform. Then, 0.5 ml of 0.1% CAA reagent was added and the volume was brought to 5 ml with acetonitrile. The absorbance was measured at 520nm against a reagent blank prepared simultaneously. The calibration graph in each instance was prepared by plotting the measured absorbance versus concentration of drug. The concentration of the unknown was read from the calibration graph or computed from the regression equation.

Analysis of Tablets

Twenty tablets were finely powdered and mixed. An accurately weighed quantity equivalent to the drug base concentration mentioned in preparation of standard solution was transferred to a 100 ml beaker, and the drug was extracted with three 20 ml portions of chloroform and filtered using a quantitative filter paper. The residue was washed with 20 ml of chloroform and the volume was finally made up to 100 ml volumetric flask with chloroform. This was further diluted to get the working solutions with chloroform. A suitable aliquot was treated as described under the preparation of calibration graphs.

Analysis of Syrups

An accurately measured volume of the syrup equivalent to 50 mg of drug base was quantitatively transferred into a 50 ml volumetric flask and made upto volume with chloroform. After diluting this 1 mg ml^{-1} solution suitably, an appropriate volume was subjected to analysis.

Results and Discussion

CAA solution in acetonitrile gave an absorption spectrum with an absorption maximum at 430 nm.

On the addition of chloroformic solution of AST or LRT to CAA solution, a bathochromic shift to longer wavelength was obtained at the room temperature (Fig 2). This new absorption band formed was the result of the formation of charge-transfer complex through the interaction of CAA as a π-acceptor and the studied drugs as n-donors followed by the formation of radical anion according to the following scheme:

$$\underset{\text{Donor}}{D} + \underset{\text{Acceptor}}{CAA} \rightleftharpoons \underset{\text{n - }\pi\text{ complex}}{[D \longrightarrow CAA]} \rightleftharpoons \underset{\text{Radical ions}}{D^{\cdot+} + CAA^{\cdot-}}$$

Formation of radical anion in such molecular interactions has been established by electron-spin resonance measurements.

Optimization of Experimental Variables

The influence of various factors on the colour development was studied to determine the optimal conditions, viz reagent concentration and choice of solvent. CAA solution in various solvents failed to give quantitative results. However, CAA in acetonitrile readily reacted stoichiometrically with the two antihistamines investigated forming a single purple CAA radical anion. Acetonitrile proved to be most suitable diluting solvent as it gave good solvating capacity for CAA, and gives the highest yield of the radical anion. Solvents such as chloroform, 2-propanol, dichloroethane, 1,4 – dioxane were not suitable because, the complex formed in these solvents either had low absorbance or was precipitated on dilution. The reaction between the drugs and CAA in acetonitrile was instantaneous and the product remained stable for at least 24 h.

Maximum absorption was obtained when 0.50 ml of 0.1% CAA solution was used in a total volume of 5 ml. Fig 3 shows the relationship between absorbance and concentration of CAA at a fixed drug concentration.

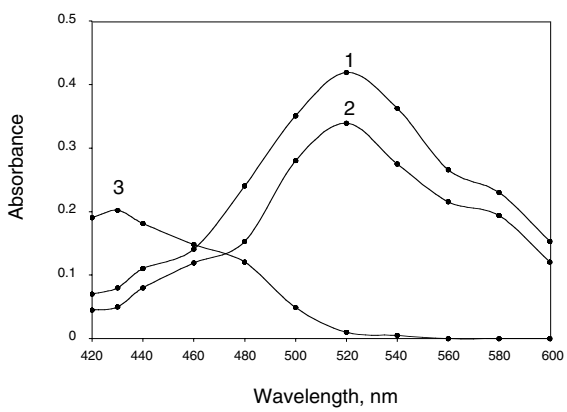

Fig 2. Absorption spectra of the CT complex formed between the drugs and CAA.
1. AST (100 μg ml^{-1}) 2. LRT (150 μg ml^{-1}) 3. Blank

Molecular Ratio and Formation Constant

The stoichiometry of the reaction between CAA and AST and LRT was studied by Job's method of continuous variations.[22] The plot (Fig 4) indicates that the interaction occurs through the formation of 1:1 (donor : acceptor) complex in both the cases. This is in agreement with the presence of a single tertiary nitrogen atom in AST and LRT molecule (Fig 1). The formation constant (K) and molar absorptivity (Œ) of the drug – CAA complex were determined by using Benesi – Hildebrand equation[23]:

$$\frac{[A_0]}{A} = \frac{1}{K}\,[D_0]\in + \frac{1}{\in}$$

where K is the formation constant, A is the absorbance and ∈ is the molar extinction coefficient of the complex, $[A_0]$ and $[D_0]$ are the initial concentration of acceptor and donor, respectively. Formation constants were obtained from the ratio of intercept to slope and ∈ from inverse intercept of

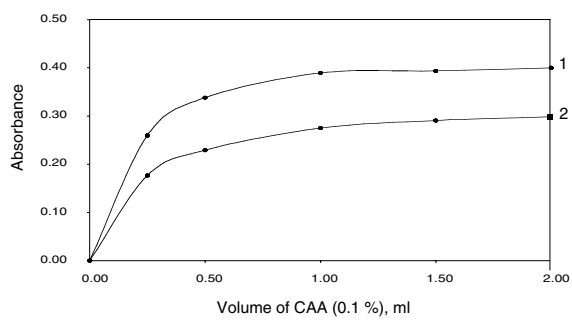

Fig 3. Effect of CAA concentration on complex formation
1. AST (80 μg ml^{-1}) 2. LRT (90 μg ml^{-1})

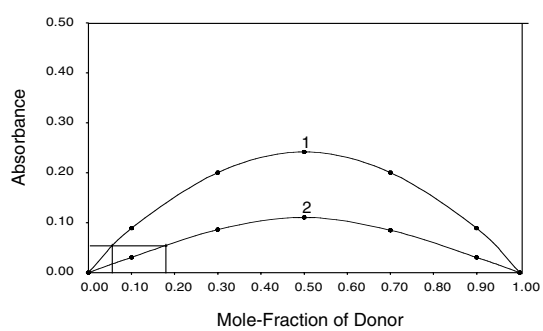

Fig 4. Job's Continuous Variation plots for LRT
1. 7. 83 x 10^{-4} M 2. 3. 91 x 10^{-4} M

Benesi – Hildebrand plots (Fig 5), which were prepared by keeping the concentration of CAA constant, and lower than the varied concentrations of the drugs as described under procedures. The results of the study are presented in Table 1.

Analytical Data

Beer's law limits, detection limits, molar absorptivity and Sandell sensitivity values are given in Table 1. Regression analysis indicated that the values of the intercept were small: –0.0128 and 0.0348 for AST and LRT respectively. Correlation coefficient values were 0.9956 and 0.9994 for AST and LRT, respectively, suggesting a perfect linearity between absorbance and concentration of drugs in the Beer's law limits studied.

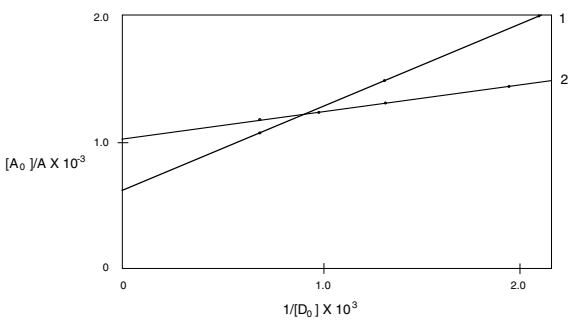

Fig 5. Benesi -Hidebrand plots of CT Complexes of AST and LRT with CAA
1. AST 2. LRT

Accuracy and Precision

The accuracy of the method was established by performing seven replicate analyses on solutions containing four different amounts (within the Beer' s law limits) of each drug and calculating the percentage error. The precision was ascertained by calculating the relative standard deviation (RSD) for seven determinations at each level. The range, percent error, standard deviation (SD), RSD (%) are given in Table 2. The comparision of the actual difference between the mean and the true value ($\bar{x} - \mu$) with the largest difference that could be expected as a result of indeterminate error ($\pm ts/\sqrt{n}$) is made in the last two columns of Table 2. It is clear from the results that at all the four levels (amounts) studied, the values of ($\bar{x} - \mu$) were less than $\pm ts/\sqrt{n}$ indicating that no significant difference existed between the mean and the true values.

Application of the Method to Formulations

The method was applied successfully to the determination of AST and LRT in their dosage forms. The results presented in Table 3 reveal that the recoveries were in the range 98.80 to 101.70 % reflecting high accuracy and precision of the method as indicated by low RSD values.

Table 1. Analytical Parameters for the Charge-Transfer Complexes of AST and LRT with CAA.

Parameter	AST	LRT
Beer's law limits, μg ml^{-1}	10 – 120	15 – 210
limit of detection, μg ml^{-1}	1.05	2.21
limit of quantification, μg ml^{-1}	3.51	7.39
Molar absorptivity, l mol^{-1}cm^{-1}	1.78 x 10^3 ψ	0.96 x 10^3 ψ
	1.66 x 10^3 ♣	1.00 x 10^3 ♣
Sandell sensitivity ng cm^{-2} per 0.001 absorbance unit	257.62	396.61
Regression equation*		
Intercept, a	-0.0128	0.0348
Slope, b	0.0042	0.0020
Confidence interval of intercept, α	-0.0128 ± 0.0350	0.0348 ± 0.0563
Confidence interval of slope, β	0.0042 ± 0.0007	0.0020 ± 0.0004
Correlation coefficient, r	0.9956	0.9994
Formation constant, K.	0.90 x 10^3 ♣	4.50 x 10^3 ♣
	1.06 x 10^3 ⊗	4.19 x 10^3 ⊗

* Y = a + bX where Y is the absorbance for concentration, X in μg ml^{-1}.
ψ : calculated from Beer's law data.
♣ : calculated from Benesi – Hildebrand plot.
⊗ : calculated from Turner and Anderson plot.

Table 2. Evaluation of Accuracy and Precision of the Method.

Antihistamine studied	Amount taken, μg ml^{-1}	Amount found*, μg ml^{-1}	Range, μg ml^{-1}	Error, %	S, μg ml^{-1}	RSD, % (n = 7)	$\bar{x} - \mu$	$\pm \frac{tS}{\sqrt{n}}$
	30.00	29.58	1.74	1.40	0.58	1.98	0.42	0.52
AST	60.00	60.59	1.98	0.98	0.93	1.54	0.59	0.83
	80.00	81.04	3.55	1.30	1.21	1.50	1.04	1.08
	120.00	121.32	4.10	1.10	1.61	1.32	1.32	1.43
LRT	150.00	150.93	2.35	0.62	1.15	0.76	0.93	1.02
	180.00	180.15	3.25	0.08	1.46	0.81	0.15	1.30

* Mean value of seven determinations at each level

$\bar{x}$ = mean value ; μ = true value ; **t** = 2.36 for n = 7 at 95% confidence level.

S = standard deviation; **RSD** = relative standard deviation

Table 3. Results of Assay of AST and LRT in their dosage forms.

Drug	Dosage form* and brand name	Label claim, mg/tablet	Found, $ mg	Mean recovery, % (n = 7)	RSD, % (n = 7)
AST	Acemiz tablet[a]	10 mg	9.88	98.80	2.07
	Acemiz syrup[a]	5 mg / 5 ml	5.05	101.00	1.21
	Alerzole tablet[b]	10 mg	10.14	101.45	1.62
	Alerzole syrup[b]	5 mg / 5 ml.	5.07	101.41	1.33
	Histeese tablet[c]	10 mg	10.17	101.70	2.03
	Histeese syrup[c]	5 mg / 5ml	5.06	101.22	1.54
LRT	Lorfast tablet[d]	10 mg	10.13	101.35	1.32
	Lormeg tablet[e]	10 mg	10.04	100.46	0.67
	Roletra tablet[f]	10 mg	9.99	99.94	0.73
	Roletra syrup[f]	5 mg / 5ml	4.98	99.69	0.68

* Marketed by : a. Lupin; b. Themis pharma; c. Micro Labs; d. Cadila pharma; e. Megacare; f. Rexcel;

$ Average of seven determinations.

Interferences

Excipients and fillers added to formulations were tested for their interference in the procedure. Fortunately, such auxillary substances as starch, talc, lactose, gelatin, magnesium stearate and sodium alginate exhibited no interferences, since in the proposed method, the free base is extracted prior to the instant complexation with CAA. This is clearly indicated from the results obtained for dosage forms (Table 3).

Conclusions

The reported method is rapid, simple and fairly sensitive. It has the advantages of a wide range of determination and high accuracy. The complex formed is stable for at least 24 h, thus permitting quantitative analysis to be carried out with good reproducibility.

References

1. Suryanarayana MV, Venkataraman S, Reddy MS, Reddy BP, Sastry CSP and Krupadanam GLD (1993) *Talanta* **40**, 1357.
2. Sadana GS and Potdar AJ (1989) *Indian Drugs* **27**, 140.
3. Sastry CSP and Naidu PY (1998) *Talanta* **45**, 795.
4. Sastry CSP and Naidu PY (1997) *Indian Drugs* **34**, 140.
5. Alwarthan AA and Al-Obaid AM (1996) *J Pharm Biomed Anal* **14**, 579.
6. Johnson R Christesen J and Lin CC (1994) *J Chromatogr Biomed Appl* **657**, 125.
7. Zhong D and Blume M (1994) *Pharmazie* **49**, 736.
8. Martens J (1995) *J Chromatogr Biomed Appl* **673**, 183.
9. Rajput SV and Vyas AG (1998) *Indian Drugs* **35**, 352.
10. Squella JA, Sturun JC Diaz MA, Perssoa H and Nunez Vergara LJ (1996) *Talanta* **43**, 2029.
11. Lin ZH (1996) *Yaowu Fenxi Zazhi* **16**, 53.
12. Mulliken RS and Person WB, *"Molecular Complexes"* (1969) Wiley – Interscience, Newyork.
13. Townshud A (1973), *Proc Soc-Anal Chem* **10**, 39; (1976) *Proc Soc-Anal Chem* **13**, 64.
14. Muralikrishna U and Krishnamurthy M (1982) *Indian J Chem* **21A**, 1018.

15. Muralikrishna U and Rao NS (1978) *Indian J Chem* **16A**, 1993.
16. Adikwu MV, Ofakansi KC and Atlama AA (1999) *Chem Pharm Bull* **47**, 463.
17. Okide GB and Odoh UE (1998) *Indian J Pharm Sci* **60**, 368.
18. Mahrons MS, Abdel salam M and Issa AS (1986) *Talanta* **33**, 185.
19. Zakhari NA, Rizk M, Ibrahim F and Walash MI (1986) *Talanta* **33**, 111.
20. Agarwal SP and Abdel Hady Elsayed M (1981) *Analyst* **106**, 1157.
21. Rizk M, Walash MI and Ibrahim FA (1981) *Analyst* **106**, 1163.
22. Rose J, "*Advanced Physico Chemical Experiments*" (1964) p 54, Pitman, London.
23. Benesi HA and Hildebrand JH (1949) *J Am Chem Soc* **71**, 2703.