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Article

In vitro study on the interaction of ketotifen fumarate with anhydrous theophylline

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The purpose of the present study was to investigate the interaction between ketotifen fumarate and anhydrous theophylline in aqueous media of various pH (1.2 and 6.8). Using Job's continuous-variation analysis and Ardon's spectrophotomeric measurement methods, the values of the stability constants of theophylline with ketotifen were determined at a fixed temperature (37 °C) at various pH. The stability constants, ranging between 5.66 and 9.92, were derived from Ardon's plot, indicating that comparatively stable complexes had formed as a result of an interaction between the drugs. However, following the interaction of theophylline with ketotifen, stability constants were <1 at gastric pH (1.2) and intestinal pH (6.8). Concurrent administration of ketotifen and theophylline could result in the formation of a stable complex and this is likely to reduce the therapeutic activities of both drugs.

Uniterms: Stability constant. Job's method. Ardon's method. Ketotifen. Theophylline.

O objetivo do presente estudo foi investigar a interação entre o fumarato de cetotifeno e a teofilina anidra em meios aquosos com vários pH (1,2 e 6,8). Utilizando a análise da variação contínua de Job e os métodos de medida espectrofotométrica de Ardon, os valores das constantes de estabilidade da teofilina com o cetotifeno foram determinados em temperatura fixa (37 oC) em vários pH. As constantes de estabilidade, variando entre 5,66 e 9,92 derivaram-se a partir do delineamento de Ardon, indicando, comparativamente, que complexos estáveis se formaram como resultado da interação entre os fármacos. Entretanto, seguindo a interação da teofilina com o cetotifeno, as constantes de estabilidade foram <1, em pH gástrico (1,2) e intestinal (8,8). A administração concomitante de cetotifeno e teofilina poderia resultar na formação de complexo estável, o que reduz a atividade terapêutica de ambos os fármacos.

Uniterms: Constante de estabilidade. Método de Job. Método de Ardon. Cetotifeno. Teofilina.

INTRODUCTION

Ketotifen is a benzocycloheptathiophene derivative that has been shown to possess anti-histaminic and anti-anaphylactic properties (Martin, Romer, 1978). It has been demonstrated that it can block the *in vitro* release of mediators from rat peritoneal mast cells (Martin, Romer, 1978). The drug inhibits the release of histamine and leukotrienes from basophils and lung tissue, antagonises histamine at H_1 receptors, inhibits calcium uptake, blocks the passive cutaneous anaphylactic reaction, reverses isoprenaline-induced beta-adrenoceptor tachyphylaxis, and inhibits both allergen-induced and drug-induced asthma (Craps, Greenwood, Radielovic, 1978). A number of clinical trials of ketotifen have shown to have a beneficial effect in the treatment of asthma (Hoshino *et al.*, 1998; Tinkelman *et al.*, 1985) equivalent to that of disodium cromoglycate, which has an established place in the treatment of asthma (B.H./M.R.C.C.T., 1972; Clarke, May, 1980). Ketotifen, which is useful in the treatment of hay fever and asthma, has been found to inhibit anaphylactic histamine release from animal tissues (Martin *et al.*, 1980). Theophylline, also known as dimethylxanthine, is a xanthine with bronchodilator properties and is used in the treatment of asthma and chronic obstructive pulmonary disease (COPD). Moreover, theophylline has been shown to have some anti-inflammatory activities, inhibiting the activity of CD4 lymphocytes *in vitro* and mediator release from mast cells (Salamzadeh *et al.*, 2008). It also inhibits bronchoconstriction produced

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by exercise and challenge testing, and has been shown to have beneficial effects on the contraction of the diaphragm, an effect which may be particularly useful in patients with COPD (Kidney *et al.*, 1990; Mak, 1997). Drug-drug interactions occur when one therapeutic agent either alters the concentration (pharmacokinetic interactions) or the biological effect (pharmacodynamic interactions) of another agent (Leucuta, Vlase, 2006). The clinical significance of a specific drug-drug interaction depends on the degree of accumulation of the substrate and the therapeutic window of the substrate (Bachmann, Lewis, 2005). The combination of theophylline and ketotifen is often used in respiratory tract infections and some have suggested that the combination is effective (Benjamin *et al.*, 1994), while others have postulated that the combination may be embryotoxic, leading to growth retardation and morphological abnormalities (Bechter, Schön, 1988). The major goal of the present study was to elucidate the possible importance of drug-drug interactions (DDIs) as a contributing factor towards drug safety and finally to observe and determine the stability of the complexes which could be formed by the interaction of ketotifen fumarate with anhydrous theophylline in aqueous media at variable pH. The values of the stability constants of theophylline with ketotifen were determined using Job's continuous-variation analysis and Ardon's spectrophotomeric measurement methods. However, following the interaction of theophylline with ketotifen, stability constants ranged between 0.4-2.0 at pH 1.2 and 4.12 at pH. 6. The stability constant values indicate that co-administration of both drugs may reduce the pharmacological effect of the drugs.

MATERIAL AND METHODS

Drugs and chemicals

Ketotifen fumarate and anhydrous theophylline were obtained from Square Pharmaceuticals Ltd., Dhaka, Bangladesh as a gift and were used without further purification. Sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate, used for the preparation of buffer solutions, were purchased from Merck, Germany. Potassium chloride, sodium hydroxide and potassium hydroxide were all of reagent grade.

Equipment

For these tests, we used a UV-Visible spectrometer (model no. UV-1600, Shimadzu, Japan), a pH meter (Mettler Toledo, Switzerland), an analytical balance (model number: AL 204-S/01) (Mettler Toledo, Switzerland), and a thermostatted water bath (Shimadzu, Japan). A Dunbuff metabolic shaking incubator (Nickel, Electrical Company, England) was used to shake the drug mixtures to attain equilibrium.

Preparation of standard solutions

Stock solutions of ketotifen fumarate ($1x10^{-3}$ M) and anhydrous theophylline($1x10^{-3}$ M) prepared by dissolving them in distilled water. These stock solutions were diluted to the desired concentration ($1x10^{-5}$ M) in the buffer solutions to obtain the working standard solutions.

Absorption spectrum analysis

In the observation of the spectra, the absorption characteristics of ketotifen fumarate and theophylline and their 1:1, 1:2, and 2:1 mixtures in the buffer solutions (Perrin, Dempsey, 1974; Mohiuddin *et al.*, 2009) at pH 1.2 and 6.8 were compared with those of each interacting species. The concentrations of the sample were kept at very dilute levels in each case and the measurements made using the UV-VIS spectrophotometer were taken at a constant temperature of the cell compartment and automatic recording unit. The stock solutions of the samples were diluted to the appropriate levels with the buffers ($1x10^{-5}$ M) at the desired pH and the spectra were recorded between 400-190 nm. The spectra were compared with those of the pure samples in each case.

Job's spectrophotometric method

According to Job's method (Job, 1971) a series of solutions were prepared in which the analytical concentration of one reactant (usually the cation) was held constant while that of the other was varied. The absorbance of a series of ketotifen fumarate with theophylline in different molar ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) was measured by keeping the total molar concentration constant. The observed absorbance of the mixtures at various molar fractions was subtracted from the sum of the values for the free drugs (ketotifen fumarate and anhydrous theophylline). The absorbance difference (D) was then plotted against the molar fractions of the drug in the mixtures. If the formation constant is reasonably favourable, two straight lines with different slopes that intersect at a molar ratio that corresponds to the ratio of drugs in the complex are obtained.

Ardon's spectrometric method

The anhydrous theophylline concentration was kept fixed ($2x10^{-4}$) while the ketotifen concentrations were

varied. The absorbance of free drug solutions and those of mixtures were measured at 300 nm at different pH. From Ardon's equation (Ardon, 1971) the values of $1/(D\text{-}C_{\varepsilon A})$ versus 1/drug were plotted and the values of the stability constants were calculated from the intercept/slope of the straight lines. In the above equation D is the absorbance of the mixture, C is the molar concentration of ketotifen, and εA is the molar extinction coefficient of the complex.

RESULTS AND DISCUSSION

By spectral observation analysis, both of the studied drugs showed absorption in the UV-VIS range. The molecular species of ketotifen fumarate and theophylline, when observed separately, showed some changes in the absorption characteristics of the drug molecules, including shifts in the absorption maxima. The initial detection of the complexation of ketotifen fumarate with theophylline was obtained from the spectra of the pure compounds as well as their 1:1, 1:2, and 2:1 mixtures in buffer solutions at pH 1.2 and 6.8 at a fixed concentration ($1x10^{-5}$ M). It is clear that each compound has a unique molecular structure or electronic configuration which is responsible for the absorption of light in the ultraviolet or visible range. For this reason, the UV-spectrum of any pure compound will be unique and totally different from another compound or a complex of that compound with another compound. The spectrum of ketotifen fumarate alone at different pH showed a sharp absorption maximum at 300 nm. When theophylline was mixed with ketotifen in a 2:1 ratio, the intensity of the peak of ketotifen changed remarkably (decreased absorbance), i.e. the absorption characteristics were altered due to the interaction, but the position of the compound did not shift. The UV spectra of ketotifen at pH 1.2 and 6.8 and the 1:1, 1:2, and 2:1 mixtures of ketotifen fumarate with theophylline at the same pH are shown in Figure 4 and Figure 5. The curves obtained by Job's method show breaks at different molar concentrations for both drugs. It was found that the curves obtained at pH 6.8 were somewhat flat compared to those at pH 1.2. On the other hand, the kinetics of the interaction between ketotifen fumarate and theophylline anhydrous were slow at pH 1.2 (Figure 1). The continuous variation plot provides information on the relative affinities of the complexes and depends on the intrinsic spectral characteristics of each complex.

Ardon's plots have been used to evaluate stability constants. It has been observed that when values of $1/(D\text{-}C_{\varepsilon A})$ are plotted against 1/drug (Figure 2), straight lines are obtained following Ardon's equation. The values of the stability constants at different pH levels are shown in

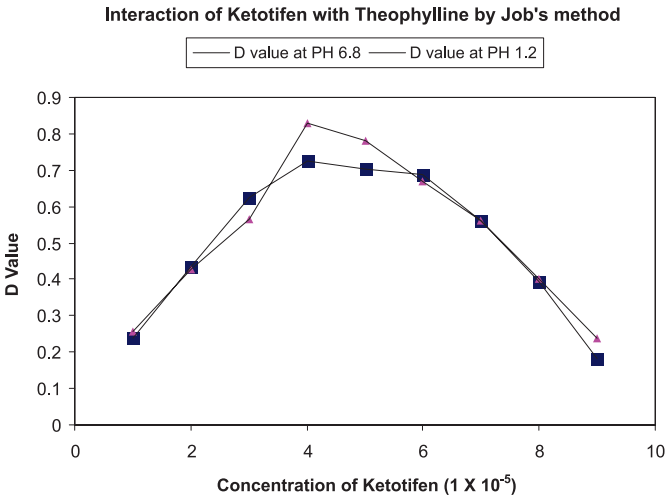

FIGURE 1 - Job's plot for the complexation of ketotifen with theophylline at 300 nm.

TABLE I - Absorbance of ketotifen at different pH (using Job's method)

Conc. of Ketotifen (M)	Absorbance (D Value)	
	pH 1.2	pH 6.8
$1x1^{0}\text{-}5$	0.256	0.288
$2x10^{-5}$	0.426	0.391
$3x10^{-5}$	0.564	0.454
$4x10^{-5}$	0.831	0.506
$5x10^{-5}$	0.78	0.514
$6x10^{-5}$	0.67	0.516
$7x10^{-5}$	0.562	0.445
$8x10^{-5}$	0.402	0.512
$9x10^{-5}$	0.237	0.448

Table III and Table V. Very low stability constant numerical values (between negative values and 1) mean that the formation of the complex due to an interaction between the drugs is readily dissociated (Table V), yielding essentially all drugs in ionic form at low pH as in the stomach (about pH 2 to 3) to as high as physiological (pH 7.4, the pH of extracellular body fluids such as serum and lymph) (Landy *et al.*, 1999).

The numerical values of the resulting stability constants were 9.92 at pH 1.2 and 5.66 at pH 6.8 when complexation occurred with ketotifen and theophylline (Table III). These values indicate a good interaction between ketotifen and theophylline. It can be assumed that these two drugs can be safely administered orally at the same time. Following Ardon's method, when theophylline is considered as the parent drug and interacts with

TABLE II - Absorbance of ketotifen at different pH (using Ardon's method, when the concentration of theophylline is constant)

1/D x 10^{-5}	1/ (D-$C_{\varepsilon A}$)	
	pH 1.2	pH 6.8
1.000	38.5	33.3
0.500	32.3	4.0
0.330	8.4	4.7
0.250	4.5	2.3
0.200	2.9	1.8
0.167	2.0	1.7
0.143	1.5	1.4

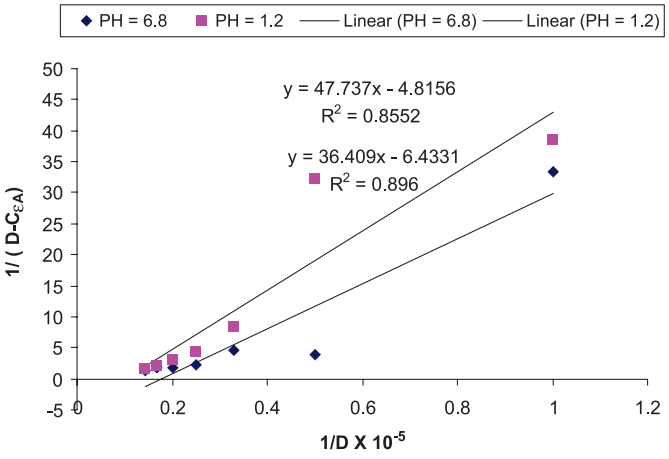

FIGURE 2 - Ardon's plot for the complexation of ketotifen with theophylline at 300 nm.

TABLE III - Stability constant of ketotifen with theophylline at different pH

System	pH	Stability constant
Interaction of ketotifen with theophylline	1.2	9.92
	6.8	5.66

TABLE IV - Absorbance of ketotifen at different pH (using Ardon's method, when the concentration of ketotifen is constant)

1/D x 10^{-5}	1/ (D-$C_{\varepsilon A}$)	
	pH 1.2	pH 6.8
1	3.226	2.577
0.500	3.125	1.724
0.333	3.300	1.754
0.250	3.367	1.698
0.200	3.497	1.761
0.167	3.546	1.715
0.143	3.145	1.698

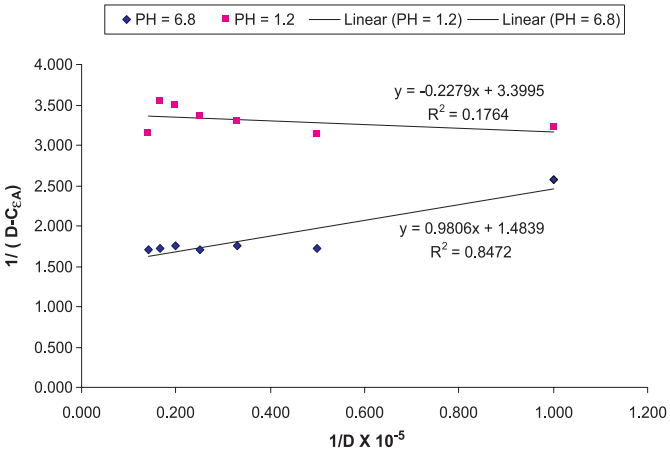

FIGURE 3 - Ardon's plot for the complexation of theophylline with ketotifen at 300 nm.

TABLE V - Stability constant of theophylline with ketotifen at different pH

System	pH	Stability constant ($1x10^{-2}$)
Interaction of theophylline with ketotifen	1.2	6.70
	6.8	66

ketotifen, a lower stability constant was found, indicating the ready solubility of both drugs and a minimal drug-drug interaction (Figure 3).

CONCLUSION

The interaction of ketotifen with theophylline decreases the free drug concentration of both drugs, which can result in decreased availability of the drugs at their receptors. Ultimately, one or both drugs may show diminished pharmacological activity. Therefore, caution should be exercised during the administration of both drugs, pending *in vivo* experiments to determine the implications of our findings.

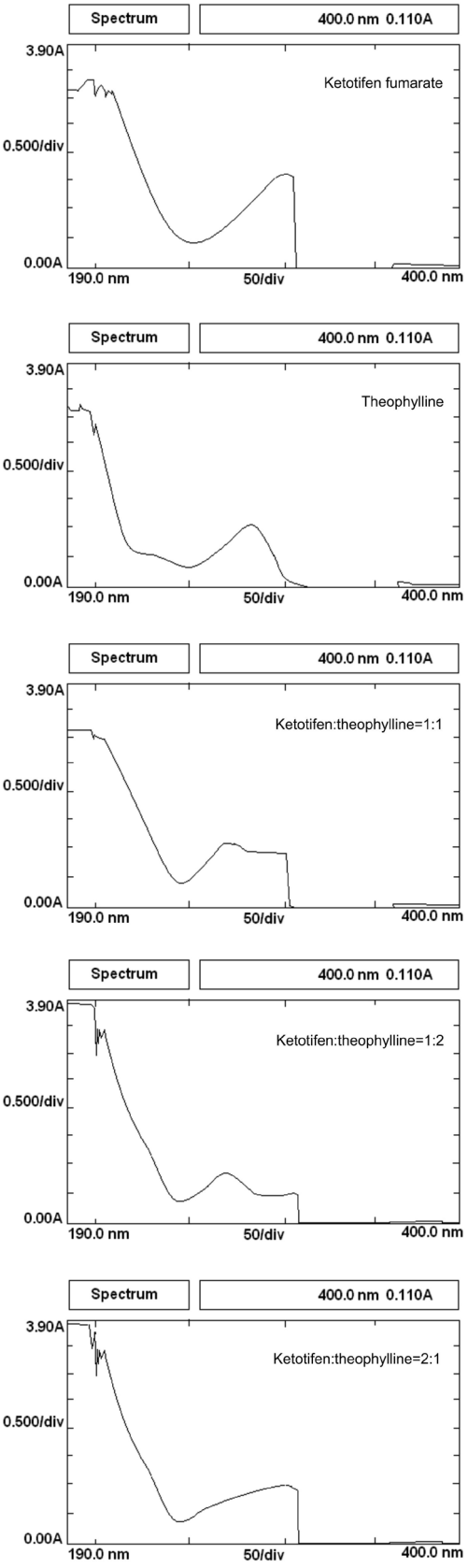

FIGURE 4 - Spectral studies of ketotifen fumarate and theophylline at pH 1.2. **X axis:** UV range; **Y axis:** absorbance.

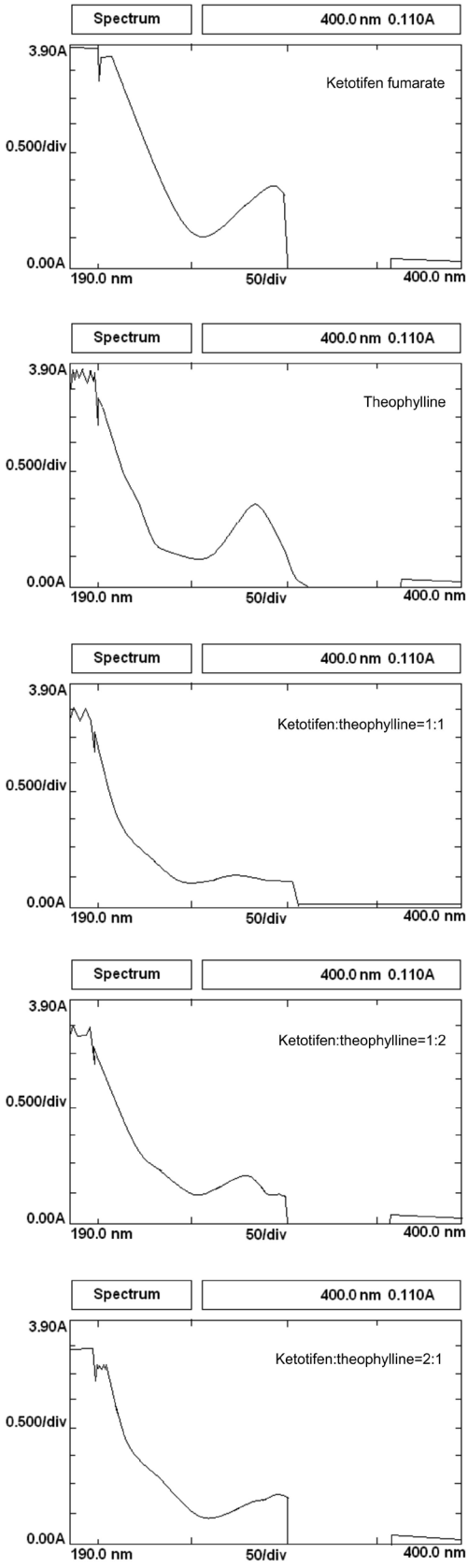

FIGURE 5 - Spectral studies of ketotifen fumarate and theophylline at pH 6.8 **X axis:** UV range; **Y axis:** absorbance.

REFERENCES

ARDON, M. Oxidation of ethanol by ceric perchlorate. *J. Chem. Soc.*, [Online]. p.1811-1815, 1957. Available at: <http: // pubs.rsc.org/en/content/articlelanding/ 1957/jr/jr 9570001811>. Accessed on: 02 aug. 2011.

BACHMANN, K.; LEWIS, J.D. Predicting inhibitory drug-drug interactions and evaluating drug interaction reports using inhibition constants. *Ann. Pharmacother.*, v.39, p.1064-1072, 2005.

BECHTER, R.; SCHÖN, H. Use of the whole-embryo culture system in drug safety assessment. *Toxicol. in vitro*, v.2, p.195-203, 1988.

BENJAMIN, V.; JACOB, A.; HAGIT, M.; MAYA, L.; ITZHAK, V. Administration of half-dose theophylline together with ketotifen to asthmatic children-a double-blind, placebo-controlled study. *J. Asthma*, v.31, p.27-34, 1994.

BROMPTON HOSPITAL. Medical Research Council Collaborative Trial. Long-term study of disodium cromoglycate in treatment of severe extrinsic or intrinsic bronchial asthma in adults. *Br. Med. J.*, v.4, p.383-388, 1972.

CHURCH, K.M.; GRADIDGE, C.F. Inhibition of histamine release from human lung *in vitro* by antihistamines and related drugs. *Br. J. Pharm.*, v.69, p.663-667, 1980.

CLARKE, C.W.; MAY, C.S. A comparison of the efficacy of ketotifen (HC 20-511) with sodium cromoglycate (SCG) in skin test positive asthma. *Br. J. Clin. Pharmacol.*, v.10, p.473-476, 1980.

CRAPS, L.; GREENWOOD, C.; RADIELOVIC, P. Clinical investigation of agents with prophylactic anti-allergic effects in bronchial asthma. *Clin. Allergy*, v.8, p.373-382, 1978.

HOSHINO, M.; NAKAMURA, Y.; SIM, J.J.; TOMIOKA, H. A comparative study of the effects of ketotifen, disodium cromoglycate, and beclomethasone dipropionate on bronchial mucosa and asthma symptoms in patients with atopic asthma. *Respir. Med.*, v.92, p.942-950, 1998.

JOB, P. Job's method of continuous variation. *Ann. Chim.*, v.9, p.113-203, 1928.

KIDNEY, J.; DOMINGUEZ, M.; TAYLOR, P.M.; ROSE, M.; CHUNG, K.F.; BARNES, P.J. Immunomodulation by theophylline in asthma. Demonstration by withdrawal of therapy. *Am. J. Respir. Crit. Care Med.*, v.151, p.1907-1914, 1995.

LEUCUTA, S.E.; VLASE, L. Pharmacokinetics and metabolic drug interactions. *Curr. Clin. Pharmacol.,* v.1, p.5-20, 2006.

MAK, V. *Chest medicine on-line, chronic obstructive pulmonary disease (COPD)*: the UK perspective. Available at: <http:// www.prioiry.com/cmol/ copd.htm>. Accessed on: 19 May 2007.

MARTIN, U.; ROMER, E. Ketotifen: a histamine release inhibitor. *Monogr. Allergy*, v.12, p.145-149, 1977.

MOHIUDDIN, M.; AZAM, Z.; AMRAN S.; HOSSAIN, M.A. In vitro study on the interaction of caffeine with gliclazide and metformin in the aqueous media. *J. Pharmacol. Toxicol.*, v.4, p.194-204, 2009.

PERRIN, D.D.; DEMPSEY, B., *Buffer for pH and metal ion control*. London: Chapman and Hall, 1974. p.211-216.

SALAMZADEH, J.; DADASHZADEH, S.; HABIBI, M.; ESTIFAIE, S. Serum and saliva theophylline levels in adult outpatients with asthma and chronic obstructive pulmonary disease (COPD): a cross-sectional study. *IJPR*, v.7, p.83-87, 2008.

TINKELMAN, D.G.; MOSS, B.A.; BUKANTZ, S.C.; SHEFFER, A.L.; DOBKEN, J.H.; CHODOSH, S.; COHEN, B.M.; ROSENTHAL, R.R.; RAPPAPORT, I.; BUCKLEY, C.E.; CHUSID, E.I.; DEUTSCH, A.J.; SETTIPANE, G.A.; BURNS, R.B.P. A multicenter trial of the prophylactic effect of ketotifen, theophylline, and placebo in atopic asthma. *J. Allergy Clin. Immunol.,* v.76, p.487-497, 1985.

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