ORIGINAL ARTICLE

IN VITRO INTERACTION STUDIES OF DILTIAZEM WITH NSAID's USING UV SPECTROPHOTOMETRY AND RP-HPLC TECHNIQUES

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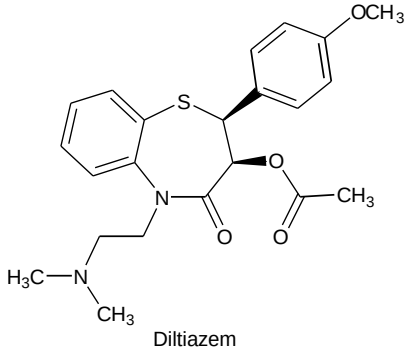
ABSTRACT

Diltiazem (DTZ) is a well-known cardiovascular drug used clinically in the treatment of angina pectoris and hypertension. Present paper deals with the *in vitro* availability studies of DTZ in presence of commonly used nonsteroidal anti-inflammatory drugs (NSAID's) like diclofenac sodium, flurbiprofen, mefenamic acid and meloxicam. Simultaneous administration of both types of drugs may alter the antihypertensive effect of DTZ. These studies were carried out using BP 2005 dissolution test apparatus in simulated human body environments at body temperature and at elevated temperature in order to study the kinetics and energitics of these interactions**.** Both the drug in each case were analyzed either by measuring the absorbance of aliquots on a UV/VIS spectrophotometer, or by RP-HPLC method. Present study clearly indicated that most of the NSAID's studied bind to DTZ forming charge transfer complexes, evident from the high availability of DTZ. Hence, concurrent administration of NSAID's with DTZ is not recommended.

Keywords: Calcium channel blocker, diltiazem-NSAID's, drug interaction.

INTRODUCTION

The calcium antagonist DTZ has been marketed for the treatment of cardiovascular disorders for more than 20 years, and it remains one of the leading products in this therapeutic area (Martindale, 2005) (Leigh *et al*., 1991). DTZ is a benzothiazepine calcium-channel blocker with peripheral and coronary vasodilatator properties, lowers blood pressure and has some effect on cardiac conduction. It is administered orally in the treatment of angina pectoris and hypertension, and may be given intravenously in the treatment of artial fibrillation or flutter and paroxysmal supraventricular tachycardia (Martindale, 2005). DTZ has, like many other drug substances, a β-dialkylaminoethyl side-chain (Leigh *et al*.,1991) (figure 1). It was originally developed as hydrochloride, but malate and maleate salts have also been described.



Diltiazem

Fig. 1

DTZ molecule consists of a benzothiazepine ring containing $OCOCH_3$, a carbonyl group, methoxyphenyl, phenyl ring and tertiary amine group. The OCH_3 substituent of the phenyl ring is positioned right over the ester and ketone groups attached to the 7-membered ring (Kojic-Prodic *et al*., 1984). The strong absorption of DTZ is due to the presence of carboxylic and carbonyl groups. The ester oxygen adds to the overall negative electrostatic field in this part of the drug molecule (Ananthanarayanan *et al.*, 1993).

Hypertension and co-existing musculoskeletal problems are two of the common conditions for which antihypertensives and analgesics as NSAID's are most commonly prescribed together (Baum *et al.*, 1985; Griffin, 1986; Roth, 1988 and Johnson, 1997). The later are a group of chemical agents that differ in their antipyretic (Styrt and Sugarman, 1990), analgesic, anti-inflammatory and inhibition of platelet aggregation activity (Vane and Botting, 1987; Williams *et al*., 1993) and are also frequently available as *over the counter* items.

Over the last two decades, there has been growing concern about probable drug interactions between NSAID's and antihypertensives. Cases with hypertensive emergency have been reported after taking piroxicam or indomethacin in patients with previously well-controlled hypertension (Oates, 1988 and Mousavy, 1991). Numerous clinical trials and some large observational studies have also been conducted to examine such an effect (Johnson *et al.*, 1993; Davidson *et al.* 1989; Wong

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et al., 1986; Koopmans *et al.,* 1984; Koopmans *et al.,* 1986; Koopmans *et al.,* 1985; Hollifield, 1976; Wright *et al.,* 1989; Gurwitz *et al.,* 1996; Reuse *et al.,* 1988; Durao *et al.,* 1977; Polonia *et al.,* 1995; Guazzi *et al.,* 1998; Jackson and Pickles, 1983; Cinquegrani and Liang, 1986; and Moore *et al.,*1981). In a study of more than 2,000 referred patients receiving NSAIDs and antihypertensive therapy, the frequency of drug interaction causing resistant hypertension was found to be about 1% annually (Johnson *et al.,* 1993). NSAIDs increase blood pressure, particularly in hypertensive subjects (Brown *et al.,* 1986, Pavlicevic *et al.,* 2005) and can partially or completely antagonize the effects of many antihypertensive drugs (Mene *et al.,* 1995); not all NSAIDs are equal in this respect. The highest elevations in mean arterial pressure are reported with indomethacin, naproxen, piroxicam and rofecoxib (Brown *et al.,* 1986), while low-dose aspirin, celecoxibe and ibuprofen, are less unwelcome in this respect. The same is true for the antihypertensive drugs, the most prone to interaction (worsening of blood pressure control) are diuretics and ACE inhibitors, and the most resistant seem to be the calcium channel blockers (Pavliceyic 2005). Several mechanisms have been demonstrated to be probably involved in the elevation of blood pressure by NSAIDs (Simons, 1995; Meade *et al.,* 1993; Wen, 1997; Garell and Matarese, 1984; MacFarlane *et al.,* 1995; Harada, *et al.,* 1998; Stillman and Schlesinger, 1990; Brater, 1979; Roberts *et al.,* 1985; Berg and Talseth, 1985; Nowalk and Wennmalm, 1981; Sahloul, 1990; Glasson *et al.,* 1979; Linz *et al.,* 1995; Blumberg *et al.,* 1977; Murthy *et al.,* 1978; and Salvetti *et al.,*1982). NSAIDs may potentially reduce the efficacy of several antihypertensive drugs (Polonia, 1997) and can also complicate the management of hypertension because of their effects on vasodilatory prostaglandins. NSAIDs, including coxibs, blunt the anti-hypertensive effects of ACE inhibitors and β-blockers in humans (Webster, 1985; Morgan and Anderson, 2003 and Mene *et al.,* 1995). By inhibiting the synthesis of prostaglandins, various NSAID's may cause sodium and water retention, worsening hypertension and causing mild edema, even in normal individuals, receiving either high or low sodium diets (Brater, 1979).

Calcium antagonists can also induce natriuresis and diuresis, which may contribute to their hypotensive activity (Krusell *et al.,* 1987 and Krishna, 1991). In normotensive and hypertensive subjects, there were no changes in the hypotensive effects of calcium antagonists when co administered with aspirin or indomethacin (Polonia *et al.,* 1995; Hardy *et al.,* 1988; Salvetti *et al.,* 1986; Webster *et al.,* 1984) (Salvetti *et al.,* 1986). Interestingly, NSAID's do not appear to counteract the anti-hypertensive effects of dihydropyridine based calcium channel blockers such as amlodipine. Studies are described which indicate that indomethacin elevates blood pressure in elderly people treated with enalapril, but not in people whose blood pressure is controlled with amlodipine or felodipine (Morgan and Anderson, 2003). Verapamil may therefore offer considerable advantages in maintaining control of blood pressure in patients who regularly receive NSAID therapy (Houston *et al.,* 1995).

Hence, simultaneous administration of DTZ with other NSAID's may affect the therapeutic effects of both types of drug. In present paper, we report interactions of DTZ with commonly used NSAID's likes diclofenac sodium, flurbiprofen, mefenamic acid and meloxicam. The energetics of these interactions were also studied.

EXPERIMENTAL

Instrumentation

The dissolution equipment was manufactured to the B.P. 2005 (British Pharmacopoeia 2005) standards. The dissolution assembly was immersed in a water bath maintained at 37 ± 0.1^0C or alternatively at 60 ± 0.1^0C. The UV/VIS (Shimadzu 1601) spectrophotometer was integrated with a PIII computer loaded with UVPC version 3.91 software. The HPLC was Shimadzu LC-10 consisting of AT VP pump and SPD-10A VP UV–VIS detector. Chromatographic system was integrated with via Communication Bus Module model CBM-102 to PIV computer loaded with a CLASS-GC software (Version 5.03) for data acquisition and mathematical calculations. Rheodyne manual injector was fitted with a 20 µl loop, a Hypersil, ODS, C-18(150 x 4.6 mm, 5 micron) column. Samples were filtered through Millipore (Millipore USA) filter 0.45µ and DGU-14 AM on-line degasser. In addition, Mettler-Toledo electronic balance, microliter syringe and micro pore filtration assembly were used in this study.

Material and reagents

DTZ was a kind gift sample from Hilton Pharmaceutical (Pvt.) Limited. Diclofenac sodium (Voren 25 mg), flurbiprofen (Froben 50 mg), meloxicam (Melfax 7.5 mg), mefenamic acid (Ponstan 250 mg) were of Yung Shin Pharmaceuticals Ind. Co. Ltd., Abbott Laboratories (Pakistan) Ltd., Ali Gohar Pharmaceuticals (Pvt.) Ltd., Parke-Davis and Co. Ltd. respectively and were purchased from the market. Reference standards of all these NSAID's were supplied by lab-9 of department of chemistry, University of Karachi. Each product was labeled and had an expiry of not less than 365 days at the time of study. All reagents used were of analytical grade from E. Merck (Germany). Methanol and phosphoric acid 85% (Merck, Germany) and HPLC grade water were used to prepare the mobile phase. Stock solutions of DTZ were prepared in mobile phase and solution of all NSAIDs was prepared in methanol. Fresh working solutions were prepared daily.

Methods

Primary solutions of 1 millimole (mMole) concentration of DTZ were prepared individually in buffers of pH 4, 7.4 and 9. From these primary solutions, stock solutions of 0.1mMole were prepared. Working standard solutions of concentration 0.005 to 0.01 mMoles were prepared by diluting the appropriate amount of stock solution in the same buffer. Absorbance of all these solutions was measured at the absorbance maxima (236 nm) against reagent blank. Beer Lambert's law was obeyed by working standard solutions at these pH, where molar absorptivities were calculated (*table* 1). In the same way, absorbances of all the working standard solutions of NSAID's were measured at their absorbance maxima against reagent blank. The maxima of diclofenac sodium was observed at 271 nm in buffer of pH 4, 276 nm in buffers of pH 7.4 and 9; flurbiprofen at 247 nm, mefenamic acid at 285 nm, and meloxicam at 362 nm.

In vitro availability of DTZ and individual NSAID's were carried out in these buffers using dissolution equipment so as to compare them after their interactions. These availability studies were carried out at 37 and 60°C so as to calculate Arrhenius parameters of these interactions.

Interaction of diltiazem with NSAID's

The interaction studies of DTZ with NSAID's were carried out at two temperatures i.e., at 37ºC to simulate human body temperature and at elevated temperature at 60ºC to see prolonged effects and to calculate ΔG, ΔH and ΔS values. Further, in order to simulate pH of GI tract as well as blood these interactions were studied at pH 4 (simulating full stomach), 7.4 and 9. These interactions could not be studied at pH 1 (simulating empty stomach) as all these NSAIDs were insoluble at this pH. In these sets of experiments, DTZ 0.03 g separately and along with individual NSAID's (diclofenac sodium 0.05g, flurbiprofen 0.1g, meloxicam 0.0075g, and mefenamic acid 0.25g) was added to the dissolution medium at zero time. Aliquots of 5 ml were withdrawn at every 15 minutes time interval for 180 minutes and assayed by UV spectrophotometer. The volume of dissolution fluid was maintained by adding an equivalent amount of dissolution fluid withdrawn, which had previously been maintained at same temperature in the same bath.

Monitoring of interaction studies by RP-HPLC

In a separate set of experiments these interactions were also monitored by high performance liquid chromatography. In these sets of experiments DTZ solutions was individually mixed with diclofenac sodium, flurbiprofen, meloxicam and mefenamic acid (each 10 ml in 100µg/ml concentration) in a 100 ml Erlenmeyer flasks. These experiments were carried out in buffers of pH 4, 7.4 and 9 at 37ºC. Each flask was allowed to stand on water bath at 37ºC for 180 minutes with continuous shaking after every 5 minutes for homogenous mixing. 3 ml sample was withdrawn at 30 minutes time interval till 180 minutes and assayed at 236nm. The chromatographic conditions were as follows:

The mobile phase consisted of methanol-water (80:20 v/v, pH 3.1 adjusted by phosphoric acid) was delivered at a flow rate of 0.5 ml/min at ambient temperature and the retention time was about 2.6 min with symmetrical peaks. The analysis was carried out under isocratic conditions. Chromatograms were recorded at 236 nm using a detector SPD-10AV VP Shimadzu UV visible.

A validated method for the analysis of DTZ in raw materials and pharmaceutical formulations by RP-HPLC was developed (Sultana et al 2007). This method was extended to the simultaneous analysis of DTZ in presence of NSAIDs. The method not only gave good resolution between DTZ and internal standard but also between DTZ and NSAIDs. The proposed method was accuarate (the accuracy results were 94.1-99.39 for DTZ recoveries), precise (the intraday and interday precision CVs were 0.035-2.2 %) and linear over the concentration range of 0.195-50 µg/ml (R^2 = 0.99954). The lower limits of detection for DTZ was found to be 0.0184µg/ml and the quantitation limit was 0.056µg/ml on the column, and therefore could be employed as a more convenient and efficient option for the analysis of DTZ and its related compounds in drug mixtures and formulations.

RESULTS AND DISCUSSION

Although it is generally believed that concomitant use of NSAIDs would counteract the effects of antihypertensive therapy in patients with hypertension or even cause hypertension in normotensive persons, there remains much controversy on the mechanisms and magnitude of such an effect (Houston, 1991; Polonia, 1997; de Leeuw, 1996). Certain antihypertensive drugs, such as Ca^{2+}-channel blockers, centrally acting alpha agonist, and diuretics seems less sensitive to antagonism by NSAIDs (Mene *et al.*, 1995, Pavlicevic *et al.*, 2005; Celis *et al.*, 2001), this is probably because their action is apparently unrelated to renal and extrarenal production of PGs (Houston 1995). Interactions between antihypertensive agents and NSAIDs are often seen in clinical practice (Bolten 2005, Pavliceyic 2005, Gurwitz *et al.*, 1994, Gurwitz *et al.*, 1996; Chrischilles and Wallace 1993). Interaction between NSAID intake and calcium channel blocker-based antihypertensive treatment in the Syst-Eur trial are reported (Celis *et al*., 2001). An interaction between diclofenac and verapamil has been demonstrated. Verapamil enhanced the anti-inflammatory effect of diclofenac *in vivo* (paw edema) and potentiated the diclofenac inhibitory effect on the chemiluminescence response of isolated human polymorphonuclear leukocytes *in vitro* (Al-Tuwaljri and Mustafa, 1992). Moreover, the long-term health impacts of such

interaction are still not clearly defined (de Leeuw, 1996). Hence, simultaneous administration of these drugs may affect the bioavailability of DTZ, which may result in the loss of therapeutic effects of drug.

i Simultaneous determination of diltiazem and NSAID's

This procedure was designed to simultaneously measure the quantities of two drugs present in the same solution, without separating them. As the λ_{max} of both the drugs was quite far apart, hence simultaneous equations (1 and 2) were developed to quantitate each drug from the solution, while countering the interference of one drug over the other.

$$C_a = \frac{A_{dilt} \cdot b_2 - A_{NSAIDs} \cdot b_1}{a_1 b_2 - a2 b_1} \quad (1)$$

$$C_b = \frac{A_{dilt} \cdot a_2 - A_{NSAIDs} \cdot a_1}{a_2 b_1 - a_1 b_2} \quad (2)$$

Where C_a and C_b were concentrations of the two components present in the solution, A was absorbance, a_1 and a_2 were the absorptivities of DTZ at λ_{max} of DTZ and λ_{max} of NSAID's, while b_1 and b_2 were the absorptivities of NSAID's at λ_{max} of DTZ and λ_{max} of NSAID's, respectively.

ii Diltiazem interactions with NSAID's

The interaction studies of DTZ with NSAID's was carried out in buffer of pH 4, 7.4 and 9 at 37 and 60°C. Prior to interaction studies of DTZ with NSAIDs the availability of DTZ alone under the conditions of experiment were investigated. Results of these studies (table 2) showed DTZ was available at all the pH studied. The results of the effects of NSAID's on the *in vitro* availability of DTZ are mentioned in tables 3-5 and are plotted in figures 2-4 wherein the differences in the extent of drug interaction has been observed.

Diclofenac sodium interactions with DTZ in buffer of pH 4, 7.4 and 9 showed a significant rise in availability of DTZ and 190, 194 and 180 % of the drug was available at the end of experiment similar behaviour was observed at accelerated temperature. When meloxicam was interacted with DTZ in buffers of pH 4, both the drugs suppressed the availability of each other and at the end of experiment both were 57 and 17 % available respectively. Availability of DTZ was significantly increased in buffers of pH 7.4 and 9 i.e., 122% and 582% respectively, at the end of experiment. There was also a rise in availability of DTZ when interacted with mefenamic acid in buffers of pH 7.4 and 9. At 37 °C in buffer of pH 7.4, mefanamic acid increased the availability of DTZ up to 211 % and at pH 9 maximum availability was 801%. At accelerated temperature availability of DTZ was tremendously increased in buffers of pH 7.4 and 9 i.e., 70703% and 620% respectively, at the end of experiment.

When flurbiprofen was interacted with DTZ in buffers of pH 4, 7.4 and 9, the availability of DTZ was significantly increased at 37°C, maximum availability was 264, 724 and 458 % in buffers of pH 4, 7.4 and 9 respectively and same trend was shown at higher temperature.

Tremendous increase in the availability of either DTZ or NSAIDs showed formation of charge-transfer complex. The resulting charge transfer complex have a higher molecular weight and hence a higher molar absorptivity. Unless the precise molecular weight of the resulting charge transfer complex and its molar absorptivity is known, the percentage of the resulting complex cannot be established. Albeit, its formation and presence is indicated by its high (> 100%) availability.

DTZ has one tertiary amine group, carbonyl group and carboxylic group which may facilitate formation of charge transfer complexes as all these sites has high density electron clouds. The probability of formation of charge transfer complex through its carboxylic group with any other drug is much more as compared to that of tertiary amine. In case of meloxicam the chances of attachment of carboxylic group with OH group are more pronounced and in case of mefanamic acid and diclofenac sodium the binding may be with secondary amine. But since flurbiprofen itself has a carboxylic group it is suggested that DTZ interacts with flurbiprofen through its tertiary amine group. The formation of charge transfer complexes between DTZ and NSAIDs are evident from the high availability values, as discussed above, and the low availability of NSAID's in presence of DTZ. This decline in availability of NSAIDs was more significant in case of diclofenac sodium and mefanamic acid in buffers of pH 4 and 7.4 (figs. 2 and 3). At accelerated temperatures same behavior was observed. All these results indicated that DTZ bonded significantly to NSAID's and formed charge transfer complexes. Moreover, temperature also favored complex formation.

Furthermore, thermodynamic studies supported these results of interaction studies. In order to study the interaction type we calculated stability constant K and thermodynamic functions (ΔG^0, ΔH^0 and ΔS^0) for DTZ NSAID's complexation in buffer of pH 4, 7.4 and 9 at 37 and 60°C was shown by Bensei-Hildebrand equation (Asker and Larose, 1987).

iii Determination of the stability constant for the diltiazem NSAID's complexation

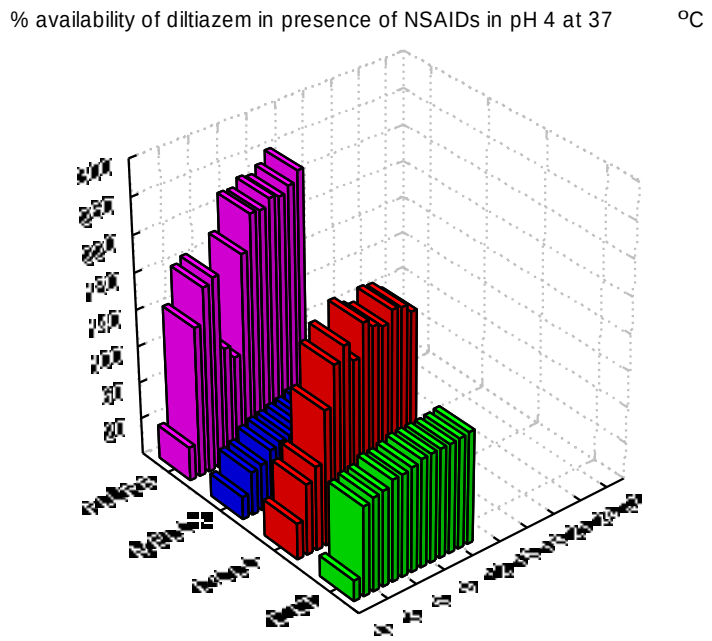

Fig. 2

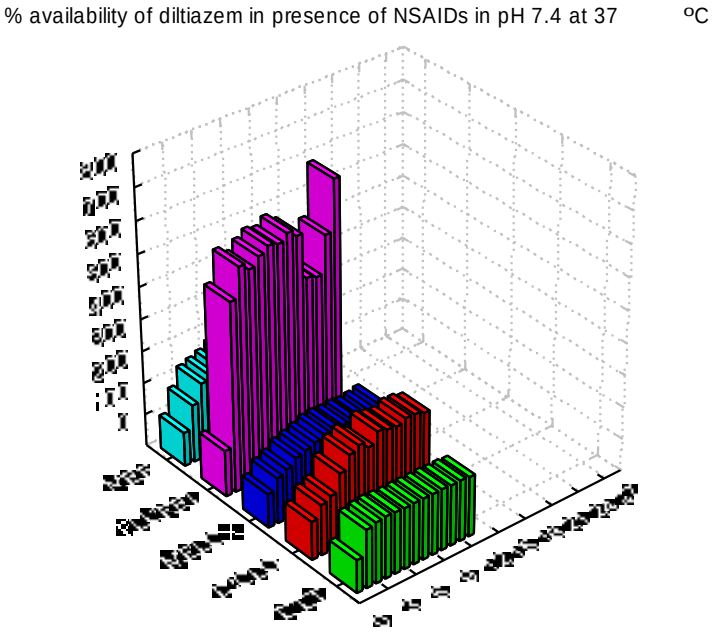

Fig. 3

In order to calculate the stability constant for DTZ-NSAIDs donor acceptor complex, Bensei-Hildebrand equation was used, which can be written as

$$A_0 / A = 1/\varepsilon + 1/ K\varepsilon \,.\, 1/D_0 \qquad (1)$$

Where, A_0 was initial concentration of acceptor, D_0 was concentration of donor, A the absorbance of the acceptor, ε molar absorptivity of the charge transfer complex at its particular wavelength and K the stability constant given in $mole^{-1}$. A plot of A_0/A versus $1/D_0$ gave the intercept of $1/\varepsilon$, while slope was given by $1/K\varepsilon$. Applying this equation we calculated stability constant K for the DTZ-NSAID's complexation which was further used to study the thermodynamic parameters for this complexation.

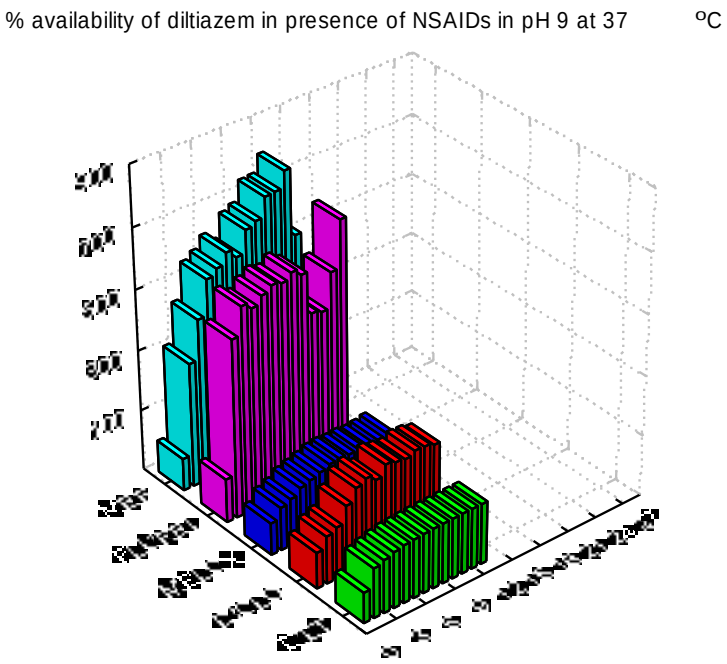

Fig. 4

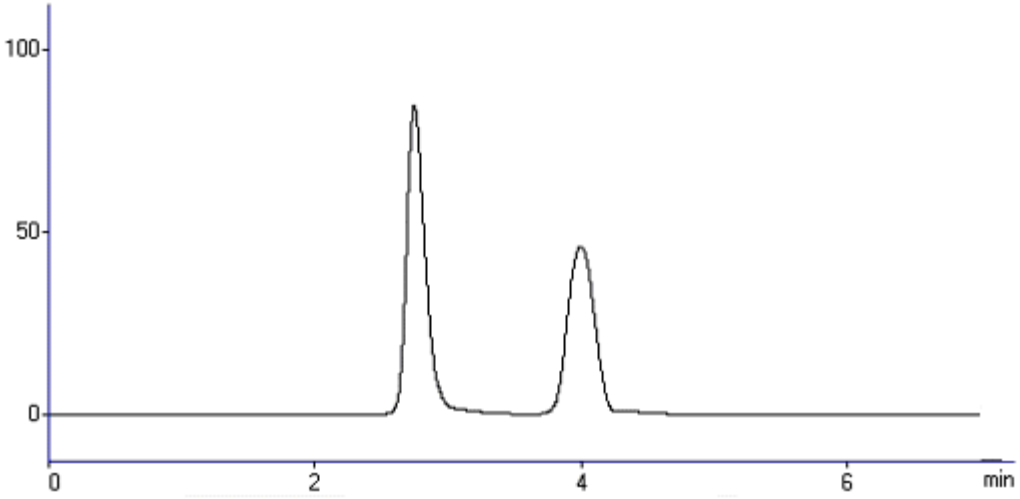

Fig. 5: A representative chromatogram showing resolution of DTZ from its internal standard. Retention time of DTZ 2.6 min; Internal standard 3.99 min

iv Thermodynamic treatment of stability constant:

The standard free energy change of complexation is related to the overall stability constant K by

$$\Delta G^o = - 2.303\ RT \log K \qquad (2)$$

Where, R is molar gas constant (1.98 cal/ mole/ deg), T is temperature. The standard enthalpy change may be obtained by Van't Hoff equation

$$\text{Log } K = - [\Delta H^0 / (2.303R)].\ 1/T + \Delta S^0 / (2.303R) \qquad (3)$$

Where $\Delta S^0/(2.303R)$ is the intercepts on the log K axis of the plot of log K versus 1/T. The standard enthalpy changes ΔS^0 is obtained from the expression

$$\Delta G^0 = \Delta H^0 - T\ \Delta S^0 \qquad (4)$$

Stability constant K and thermodynamic functions ΔG°, ΔH° and ΔS° for DTZ and NSAID's complexation in buffer of pH 4, 7.4 and 9 are given in table 5. The results

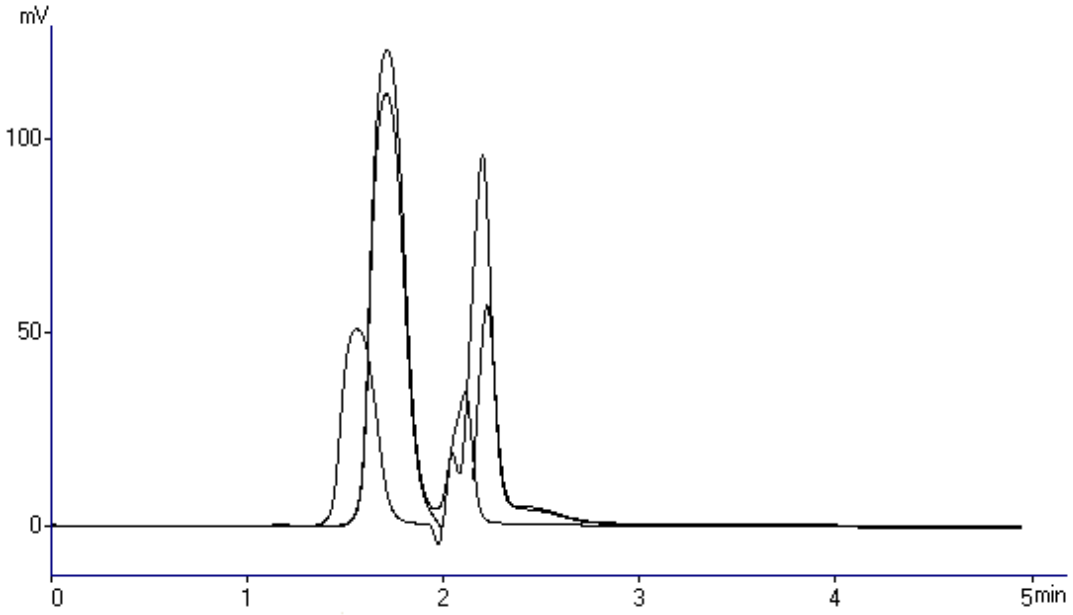

Fig. 6: Chromatograms showing drift in retention time as well as change in AUC of DTZ in presence of diclofenac sodium, meloxicam and flurbiprofen.

of standard Gibb's free energy values showed that the reaction between DTZ and NSAID's was spontaneous. In buffer of pH 4 and 9 when DTZ interacted with diclofenac sodium and flurbiprofen, enthalpy (ΔH°) and entropy (ΔS°) values indicated that their donor acceptor complex was formed due to vander Waal or hydrogen bonding. In case of meloxicam, resultant thermodynamic functions showed that chelation caused complex formation, whereas in case of mefenamic acid there were hydrophobic interaction. Likewise, diclofenac sodfium in pH 7.4 and flurbiprofen in pH 4 and 7.4 also showed positive signs of entropy and enthalpy, which indicated that complex was formed due to hydrophobic interaction. Hydrophobic binding phenomenon was first proposed by Kauzmann (Kauzmann, 1959) in which the tendency of hydrophobic part of molecules avoids water because they are not readily accommodated in the hydrogen-bonding structure of water. This interaction involves Vander Waal forces, hydrogen bonding of water molecules in a three-dimensional structure, and other interactions.

HPLC Determination of diltiazem

HPLC was successfully employed for the analysis of DTZ in presence of NSAIDs in buffers of pH 4, 7.4, and 9 at ambient temperature. There was a good separation and resolution of the chromatographic peaks of DTZ as well as NSAIDs. DTZ was quantitated by measuring their area under curve (AUC) and % recovery. In case of DTZ-NSAID interactions considerable drift in retention time of DTZ was observed. A representative chromatogram showing separation of DTZ from internal standard is shown in figures 5, while figure 6 shows that after interaction the NSAIDs complexes of DTZ appeared at 1-2 minutes as compared to reference (2.6 min). The results of the effects of NSAID's on the recovery of DTZ are mentioned in table 7.

Table 1: Epsilon value of diltiazem and NSAIDs at various pH level at 37 ^{0}C

Drugs	Epsilon(ε moles^{-1}Lcm^{-1})		
	pH 4	pH 7.4	pH 9
Diltiazem	23709	25128	22920
Diclofenac sodium	9237	10173	9804
Flurbiprofen	19740	18591	21650
Meloxicam	13904	12452	13154
Mefenamic acid	-	7239	7511

Table 2: Availability of diltiazem at various pH levels at 37°C.

Time (min.)	% availability		
	pH 4	pH 7.4	pH 9
0	2.70	3.03	1.34
15	78.1	82.40	65.5
30	82.4	90.29	76.4
45	84.6	93.89	86.1
60	91.5	92.52	92.6
75	91.9	90.49	95.1
90	94.8	91.85	97.5
105	98.7	92.65	99.1
120	99.4	93.27	99.9
135	99.8	95.26	100.2
150	99.9	97.94	101.3
165	101.2	98.59	101.1
180	103.1	98.84	101.3

Table 3: Concentration of diltiazem (%) in presence of NSAIDs in buffer of pH 4.

Time (Min)	Diclefenac sodium		Meloxicam		Flurbiprofen	
	37 ^{0}C	60 ^{0}C	37 ^{0}C	60 ^{0}C	37 ^{0}C	60 ^{0}C
0	19.57	5.61	4.78	4.22	16.81	35.21
15	57.68	63.75	27.22	47.96	144.24	217.62
30	71.79	87.26	30.90	58.41	183.10	343.69
45	127.79	118.33	42.15	76.42	189.32	277.07
60	171.30	134.64	46.72	86.98	107.76	320.78
75	186.62	151.46	49.01	93.92	93.65	364.89
90	166.30	152.95	52.19	98.91	201.05	358.68
105	201.48	193.18	54.80	112.51	239.59	430.17
120	193.06	229.83	59.59	116.45	238.89	398.09
135	188.56	212.30	58.42	117.65	247.52	451.68
150	201.46	217.14	60.15	119.61	244.91	447.86
165	199.61	207.82	60.91	117.31	252.45	447.50
180	190.38	207.95	57.23	112.31	264.34	485.96

Table 4: Concentration of diltiazem (%) in presence of NSAIDs in buffer of pH 7.4.

Time (Min)	Diclofenac sodium		Meloxicam		Flurbiprofen		Mefenamic acid	
	37 ^{0}C	60 ^{0}C	37 ^{0}C	60 ^{0}C	37 ^{0}C	60 ^{0}C	37 ^{0}C	60 ^{0}C
0	19.57	2.77	5.94	6.74	44.36	10.84	5.70	3266.15
15	57.68	61.43	42.45	51.75	490.80	518.78	79.94	35267.81
30	71.79	107.34	56.18	59.49	583.21	546.74	135.80	43776.46
45	127.79	129.02	77.94	60.07	562.68	560.78	146.89	51155.96
60	171.30	131.04	87.95	64.96	589.53	518.20	158.52	58164.05
75	186.62	134.78	98.81	70.80	608.29	579.51	178.95	54094.87
90	166.30	141.68	111.08	73.35	601.14	627.91	183.22	56234.40
105	201.48	157.86	115.40	80.68	621.95	590.92	187.02	62349.45
120	193.06	155.89	112.92	94.05	600.92	613.04	202.68	63272.04
135	188.56	170.85	116.06	94.64	462.68	658.89	227.31	68248.14
150	201.46	177.27	112.37	95.08	453.08	647.23	211.24	62828.85
165	199.61	178.23	116.31	96.26	568.37	629.74	227.31	68088.46
180	190.38	197.25	122.92	97.02	724.00	674.69	211.24	70703.56

When DTZ is simultaneously given with diclofenac sodium in buffers of pH 4, 7.4 and 9, the recovery of DTZ showed that after an interval of 90 minutes, 99, 103 and 111% of the drug was present in the solution respectively and after 180 minutes 124, 121 and 125% of the drug could be recovered. When mefenamic acid interacted with DTZ in buffers of pH 7.4 and 9, 89 and 91% of the drug was present respectively after half interval of the experiment and at the end of experiment, 92 and 93% was there in the medium.

From the results listed in table 7, it is quite apparent that the recovery of DTZ was significantly increased in presence of flurbiprofen. 239, 257 and 250% of the drug was present in buffers of pH 4, 7.4 and 9 respectively after an interval of 90 minutes and 250, 292 and 264% of the drug was recovered at end of the experiment. This higher drug content is also indicative of formation of flurbiprofen complex. There was also a rise in the recovery of DTZ when interacted with meloxicam in buffers of pH 4, 7.4 and 9. At half of the time 221, 219 and 216% was recovered respectively and after 180 minutes 238, 227 and 228% of the drug could be recovered.

Interactions of DTZ in presence of NSAIDs revealed that the recovery of DTZ increased which reflects its complexation with this drug when these drug –drug interactions were carried out at different pH.
The results of this interaction also proved a drastic change in availability of DTZ in presence of NSAIDs. Hence, it is suggested from the above findings and results that combined use of NSAIDs with DTZ is highly not recommended and should be avoided.

CONCLUSION

Simple analytical procedures using spectrophotometer and RP-HPLC connected with UV detection has been described for the determination of DTZ in presence of NSAIDs. Results of the present study indicated that all NSAIDs studied bind to DTZ, and hence altered its availability due to strong drug-drug interaction. These interactions with flurbiprofen and meloxicam were more significant. There were many evidences for these interactions, a high absorptivity showing > 100% availability, a drift in retention time in HPLC and results of thermodynamic data showed that a charge transfer complex formation took place. Hence, it is recommended that DTZ should not be coadministered with NSAIDs in high-risk patients.

ACKNOWLEDGEMENT

The authors are thankful to Higher Education Commission for financial assistance of the project.

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Table 5: Concentration of diltiazem (%) in presence of NSAIDs in buffer of pH 9.

Time (Min)	Diclefenac sodium		Meloxicam		Flurbiprofen		Mefenamic acid	
	37 ^{0}C	60 ^{0}C	37 ^{0}C	60 ^{0}C	37 ^{0}C	60 ^{0}C	37 ^{0}C	60 ^{0}C
0	19.57	2.77	5.94	6.74	44.36	10.84	6.78	20.60
15	57.68	61.43	42.45	51.75	490.80	518.78	311.24	239.75
30	71.79	107.34	56.18	59.49	583.21	546.74	442.34	357.89
45	127.79	129.02	77.94	60.07	562.68	560.78	559.83	407.93
60	171.30	131.04	87.95	64.96	589.53	518.20	581.14	487.03
75	186.62	134.78	98.81	70.80	608.29	579.51	618.65	541.09
90	166.30	141.68	111.08	73.35	601.14	627.91	590.17	564.37
105	201.48	157.86	115.40	80.68	621.95	590.92	664.86	575.84
120	193.06	155.89	112.92	94.05	600.92	613.04	679.62	584.09
135	188.56	170.85	116.06	94.64	462.68	658.89	744.16	597.46
150	201.46	177.27	112.37	95.08	453.08	647.23	747.21	614.40
165	199.61	178.23	116.31	96.26	568.37	629.74	801.50	631.32
180	190.38	197.25	122.92	97.02	724.00	674.69	581.86	620.35

Table 6: Thermodynamic data of diltiazem on interaction with NSAIDs.

	Temp K	Diclofenac sodium			Meloxicam			Flurbiprofen			Mefenamic acid	
		pH 4	pH 7.4	pH 9	pH 4	pH 7.4	pH 9	pH 4	pH 7.4	pH 9	pH 7.4	pH 9
K	310	3461	933975	556	96304	1.000	255	3260	302	67.4	515	2558
	333	6855	1200.5	3707	79007	9838	2489	2997	524	820	2.79	348.7
ΔG	310	-500	-8440	-3880	-7567	-0.004	-3402	-4966	-3506	-2585	-38833	-4817
	333	-582	-4352	-5045	-6923	-6063	-5156	-5269	-4129	-4425	-677	-3861
ΔH	310	-6073	46373	-16867	-1760	-0.8504	-0.21	757	25412	-2221	43174	16472
	333	-6524	49814	-18118	-1890	-0.7916	-0.19	747	27299	-2386	0.41826	1766
ΔS\\	310	-6057	46400	-1685	-1735	-0.846	-10.7	773	25424	-2220	43186	16487
	333	-6506	49827	-18103	-1869	-17.4	-15.2	763	27311	-2385	2.309	17707

Table 7: (%) Recovery of diltiazem in presence of NSAIDs by the RP-HPLC method.

Time (min)	Diclofenac sodium			Meloxicam			Flurbiprofen			Mefenamic acid	
	pH 4	pH7.4	pH 9	pH 4	pH7.4	pH 9	pH 4	pH7.4	pH 9	pH7.4	pH 9
30	92.78	98.96	96.54	212	207.59	202.8	235.8	242.3	228.4	84.87	87.16
60	98.4	100.18	98.06	218	218.6	214.7	235.9	252.9	238.16	88.63	88.22
90	98.7	103.08	111.88	220.9	218.76	216.46	239.3	256.9	250.4	89.38	91.02
120	101	104.3	112.7	225.8	219.04	221.01	236.1	278.5	255.6	92.04	91.88
150	112.4	104.3	116.9	236.2	219.5	228.11	245.4	284	261.22	92.22	92.5
180	123.7	120.6	125.39	237.7	226.8	228.25	249.9	292	264.3	92.24	93.2

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