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Original Article

Chromatographic Release Profile of Active Pharmaceutical Ingredients of Synthesized Prodrug and Codrug of Aspirin+Paracetamol and Indomethacin+Paracetamol in Physiological Fluids

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ABSTRACT

Prodrug is the precursor of drug which is made by derivatization of the same to enhance the bioavailability by pharmacokinetics, lipid solubility by partition coefficient and increase the physicochemical and biochemical parameters by pharmacodynamics. All two prodrugs showed different logP values and molecular weights according to the solubility parameters and electronegativity: logP profile: Prodrug-B>Prodrug-A; molecular weight profile: Prodrug-B>Prodrug-A.

The main side effect of NSAID is gastric acidity due to release of free H+ because all NSAIDs have free-COOH (carboxylic acid) group which act by competitive inhibition of cyclooxygenase enzyme (COX1/COX2). Here the target of this project has been designed in such a way to convert the free –COOH/–OH of API (aspirin/paracetamol) into prodrug of two ester (–COO–) and one amide (–CONH–) linkage (Prodrug-A) and one ester and two amide linkage (Prodrug-B) which releases free API after metabolic hydrolysis in acidic pH: 1-4 and alkaline pH: 7-9. Since the prodrugs are repository forms so chances to release gastric acid has been minimized due to non-availability of free-COOH group in stomach. The biotransformation of active drug from prodrug takes such a time in stomach that all goes upto duodenum and then ileum of small intestine that chances of acidity is reduced. Finally all prodrugs go to small intestine where alkaline pH starts so gastric acidity is reduced. Since all two prodrugs are made of two NSAID: Prodrug-A (logP=2.15) releases Aspirin and Paracetamol, Prodrug-B (logP=3.94) releases Indomethacin and Paracetamol which shows distinct two Rt values in HPLC both in acidic an alkaline hydrolysis and these Rt values of Prodrugs match with the individual API components so the purpose of our goal has been completed successfully. The pH of gastric acid varies from 1.5-3.5 in the human stomach lumen, the acidity being maintained by the proton pump H+/K+ ATPase. So the pattern for acid hydrolysis was adjusted at pH=3-3.5 by HCl. The pH of intestine varies from 5.6-6.9, so the pattern for alkaline hydrolysis was adjusted at pH=7.0-8.0 by NaOH. In case of codrug which is made by non-covalent interactions such as hydrogen bonding, ionic interactions, Van der Waals interactions and π-interactions between two APIs, so the release of parent molecule will be faster than prodrug both in acidic as well as in alkaline pH because prodrug is made by covalent bonding between two APIs.

log P profile: Codrug-B (3.42)>Codrug-A (1.55); molecular weight profile: Codrug-B (508.94g)>Codrug-A (331.31g).

Keywords: API, Mobile phase, Rt-HPLC, Covalent bond, Acidic hydrolysis, Alkaline hydrolysis, Hydrogen bonding, Ionic interactions, Van der Waals interactions, π-interactions.

INTRODUCTION

Hydrolysis Pattern of Prodrug-A

Ester linkage of Prodrug–A is ruptured by acid/alkali to free API (Aspirin and Paracetamol) by hydrolytic cleavage (Figure 1).

Esters and amides are two linkages which are hydrolysable to produce two separete entities. Esters produce carboxylic acid moiety (–COOH) and alcohol/phenol moiety (–OH) whereas amides produce carboxylic acid (–COOH) and amino moiety ($–NH_2$). Nitriles or cyanides (–CN) are also hydrolysable group but this is not a linkage but this is a functional group attached at the end terminal which is hydrolysed into carboxylic acid (–COOH) and free amino group ($–NH_2$). Hydrolysis is possible in acidic and alkaline medium which ruptures the linkages to produce the desired moiety.

Our prodrug is formed by covalent bonding between two APIs to produce Prodrug-A made by aspirin and paracetamol which produce two ester and one amide linkage whereas Prodrug-B is made by indomethacin and paracetamol which produce one ester and two amide linkages[1,2].

Hydrolysis Pattern of Prodrug-B

Ester linkage of Prodrug-B is ruptured by acid/alkali to free API (Indomethacin and Paracetamol) by hydrolytic cleavage (Figure 2).

Actually acyl [–O–CO–] and ester [–CO–O–] both have same linkages so Prodrug-A have two ester and one amide linkages so logP=2.15 whereas Prodrug-B has one ester [–CO–O–] and two amide [–CO–NH–] linkages so logP=3.94, because the ester and amide both are susceptible for hydrolysis but in amide (–CONH–) three lone pairs of electrons are present (two for oxygen and one for nitrogen) and in ester (–COO–) four lone pairs of electrons are present (four for two oxygen). So the electron density of ester is greater than amide and electronegativity of oxygen is 3.44 and for nitrogen is 3.04. So total electronegativity of ester (–COO–) is 3.44+3.44=6.88 and for amide (–CONH–) is 3.44+3.04=6.44. Hence ester is more susceptible for hydrolysis compared to amide.

Release Pattern of Codrug-A

Non-covalent interactions such as hydrogen bonding, ionic interactions, Van der Waals interaction and π-interaction are ruptured to release free API (Aspirin and Paracetamol) during hydrolytic cleavage (Figure 3).

Lone pair profile: Ester>Amide and Electronegativity profile: Ester>Amide. In this case Prodrug-A: two ester and one amide, so lone pair profile: 4×2=6 for ester and 3 for amide; hence 6 (ester electronegativity)>3(amide electronegativity) for Prodrug-A (ester is greater than amide so logP is 2.15). In case of Prodrug-B: one ester and two amide, so lone pair: 4 for ester and 3×2=6 for amide; hence 6 (amide electronegativity)>4 (ester electronegativity) for Prodrug-B (Amide is greater than ester so logP is 3.94). Hence Prodrug-B is more nonpolar than Prodrug-A[3,4].

Release Pattern of Codrug-B

Non-covalent interactions such as hydrogen bonding, ionic interactions, Van der Waals interactions and π-interactions are ruptured to release free API (Indomethacin and Paracetamol) during hydrolytic cleavage (Figure 4).

Prodrug-A and Prodrug-B both have ester linkages which is ruptured in acidic and alkaline pH into two different moieties. Prodrug-A produces aspirin and paracetamol and Prodrug-B produces indomethacin and paracetamol. These two products produce two peaks at HPLC to show different R_t values according to their polarity followed by logP: Aspirin (1.19), Indomethacin (3.10) and Paracetamol (0.34). Since the prodrugs are made by covalent linkages of esters and amides so it takes some time to be ruptured into desired API according to the polarity whereas in Codrug-A and Codrug-B which are made by non-covalent interactions such as hydrogen bonding, ionic interactions, Van der Waals interactions and π-interactions between two APIs, so the release of parent molecule is faster than prodrug both in acidic as well as in alkaline pH because prodrug is made by covalent bonding between two APIs[5-7].

Selection of Ratio of Mobile Phase

The solution containing 100 µg/ml of Prodrug-A, Prodrug-B, Codrug-A and Codrug-B respetively was chromatographed with mobile phase of different ratio of methanol and water.

EXPERIMENTAL

Reagents and Materials, Prodrug-A synthesized in our college lab, Methanol (HPLC grade, Finar Chemicals Ltd, Ahmedabad, India), Water

(HPLC grade, Finar Chemicals Ltd, Ahmedabad, India).

Equipments and Instruments

Shimadzu HPLC instrument (LC-2010 CHT) equipped with prominence diode array detector (SPD-M20A) (Software LC Solution), Analytical balance (Acculab ALC-2014, Huntingdon Valley, PA), Ultra sonicator (EN 30 US, Enertech Fast Clean, Mumbai, India), Hot air oven (TO-90S, Thermolab, Mumbai, India), pH meter (Thermo Electron Corp., Pune, India) (Table 1).

A. Aspirin profile: Standard aspirin at 284 nm in mobile phase ACN: Triethylamine buffer (50:50 v/v):-(logP=1.19). Aspirin releases moderately slow (R_t=2.3 min) because it's logP is 1.19 (semipolar) (Figure 5).

B. Indomethacin profile: Standard Indomethacin peak at 278 nm in mobile phase Water: ACN (80:20 v/v):- (logP=3.10). Indomethacin releases slow (R_t=5.5 min) because it's logP is 3.97 (nonpolar) (Figure 6).

C. Paracetamol profile: Standard paracetamol peak at 230 nm in mobile phase. Phosphate Buffer: ACN (40:60 v/v):- (logP=0.34). Paracetamol releases first (R_t=2 min) because it's logP is 0.34 (highly polar) (Figure 7).

Prodrug-A Profile (logP=2.15)

Prodrug-A has logP 2.15 so it releases slowly (R_t=5.5 min) due to nonpolar nature (Figure 8).

Paracetamol has R_t=2.75 min in acidic medium and 2.8 min in alkaline medium; aspirin has R_t=4 min in acidic medium and 3.8min in alkaline medium (Figure 9).

Prodrug-B Profile (logP=3.94)

Prodrug–B has logP 3.94 and R_t=6.8 min so release rate is low (Figure 10).

Paracetamol has R_t=2.5 min in acidic medium and 3.5 min in alkaline medium; indomethacin has R_t=6.35 min in acidic medium and 6.15 min in alkaline medium (Figure 11).

Codrug-A Profile

Codrug-A has logP 1.55 so it releases fast due to polar nature. Codrug-A has logP 1.55 so it is polar in nature so it shows R_t=3.4 mins (Figure 12).

Paracetamol has R_t=2.75 min in acidic medium and 2.8 min in alkaline medium; aspirin has R_t=4 min in acidic medium and 3.8 min in alkaline medium (Figure 13).

CODRUG-B PROFILE

Codrug-B has logP 3.42 so it releases slow due to nonpolar nature.

Prodrug-B is highly nonpolar so it releases at R_t 6.8 min (Figures 14 and 15).

Paracetamol has R_t=3.4 min in acidic medium and 3.4min in alkaline medium; indomethacin has R_t=6.8 min in acidic medium 6.35 min in alkaline medium (Figures 16 and 17).

CONCLUSION

API Profile

Aspirin releases moderately slow (R_t=3.3 min) because it's logP is 1.19 (semipolar). Indomethacin releases slow (R_t=6.8 min) because it's logP is 3.97 (nonpolar). Paracetamol releases first (R_t=2.5 min) because it's logP is 0.34 (highly polar).

Codrug-A has logP 1.55 so it is polar in nature so it shows Rt=1.8 mins (Figure 12).

Prodrug Profile

Prodrug-A has logP 2.15 so it releases slowly due to nonpolar nature. Prodrug-A has R_t=5.5 min. Paracetamol has R_t=2.75 min in acidic medium and 2.8 min in alkaline medium; Aspirin has R_t=4 min in acidic medium and 3.8 min in alkaline medium. Prodrug-B has R_t 6.8 min. Prodrug-B has logP 3.94 so release rate is slower. Paracetamol has R_t=2.5 min in acidic medium and 3.5 min in alkaline medium; Indomethacin has R_t=6.35 min in acidic medium and 6.15 min in alkaline medium.

Codrug Profile

Codrug-A has R_t=3.4 min and logP 1.55. Paracetamol has R_t=2.75 min in acidic medium and 2.8 min in alkaline medium; Aspirin has R_t=4 min in acidic medium and 3.8 min in alkaline medium. Codrug-B has logP 3.42. Paracetamol has R_t=3.4 min in acidic medium and 3.4 min in alkaline medium; Indomethacin has R_t=6.8 min in acidic medium 6.35 min in alkaline medium.

logP profile: Prodrug-B (3.94)>Codrug-B (3.42)>Prodrug-A (2.15)>Codrug-A (1.55).

R_f profile: Prodrug-B (6.2)>Codrug-B (5.6)>Indomethacin (5.2)>Prodrug-A (4.8)>Codrug-A (4.2)>Aspirin (3.7)>Paracetamol (2.8).

R_t profile: Prodrug-B (6.8 min)>Codrug-B (6.38 min)>Indomethacin (5.5 min)>Prodrug-A (5.5 min)>Codrug-A (3.4 min)>Aspirin (2.3 min)>Paracetamol (2 min).

ACKNOWLEDGEMENT

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Chromatographic release profile of active pharmaceutical ingredients of synthesized prodrug and codrug of aspirin+paracetamol and indomethacin+paracetamol in physiological fluids is the outcome of the total efforts and applications of B.Pharm. course contents into practical approach. The authors are thankful to Shri Sarvajanik Pharmacy College, Mehsana for providing drugs and laboratory facilities to perform synthesis of prodrug and codrug with their analytical profiles to fulfill this project with grand success. The authors are thankful to quality assurance lab of Shri Sarvajanik Pharmacy College, Mehsana for TLC and HPLC studies respectively.

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	Trials	Ratio	Remark		Trials	Ratio	Remark
Prodrug-A/ Codrug-A	1	Methanol: Water (60:40)	Tailing	**Prodrug-B/ Codrug-B**	1	ACN: Water (80:20)	Tailing
	2	Methanol: Water (70:30)	Tailing		2	ACN: Water (70:30)	Tailing
	3	ACN: Water (60:40)	Tailing		3	ACN: Methanol (80:20)	Tailing
	4	ACN: Water (70:30)	Tailing		4	ACN: Methanol (70:30)	Tailing
	5	Methanol: Water (80:20)	**Symmetrical peak**		5	Methanol: Water (80:20)	Tailing
					6	Methanol: Water (70:30)	**Symmetrical peak**

Table 1. Selection of mobile phase for Prodrug-A, Prodrug-B, Codrug-A and Codrug-B

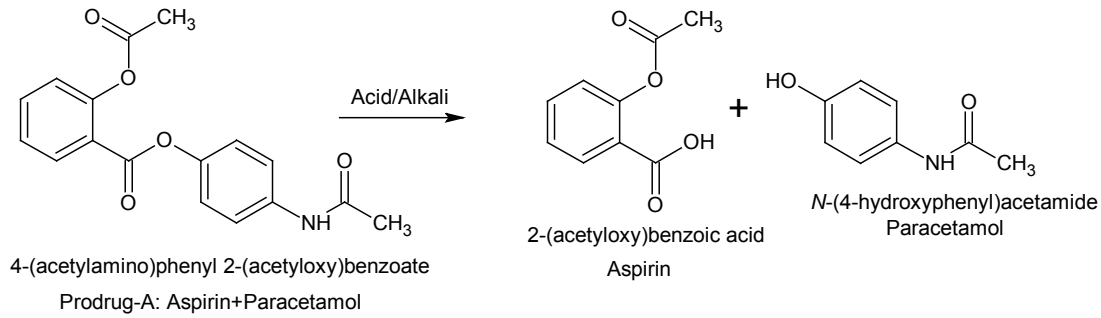

Figure 1. Hydrolysis pattern of Prodrug-A

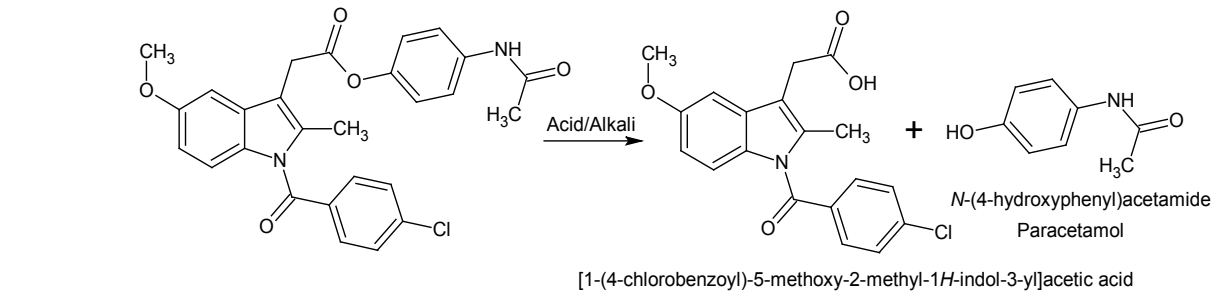

Figure 2. Hydrolysis pattern of Prodrug-B

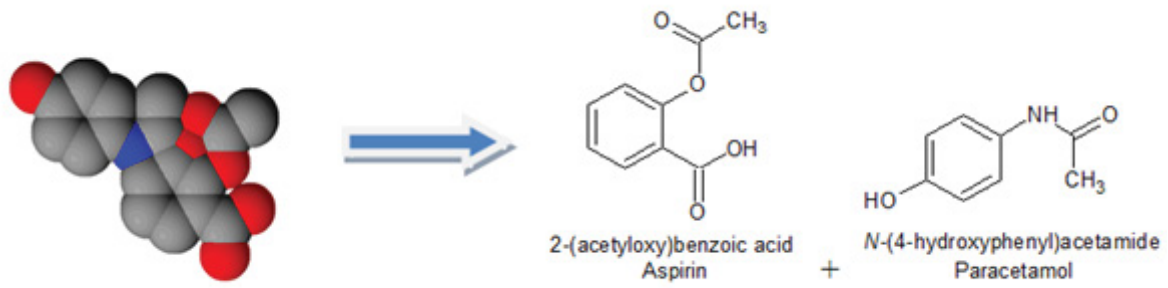

Figure 3. Release pattern of Codrug-A

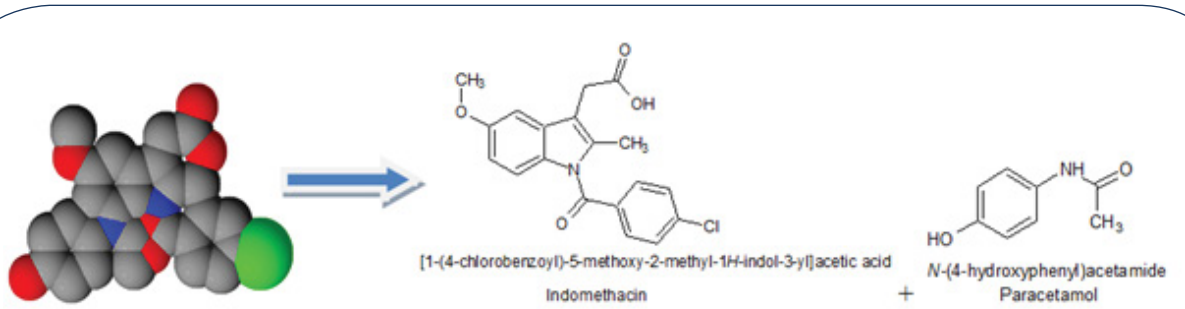

Figure 4. Release pattern of Codrug-B

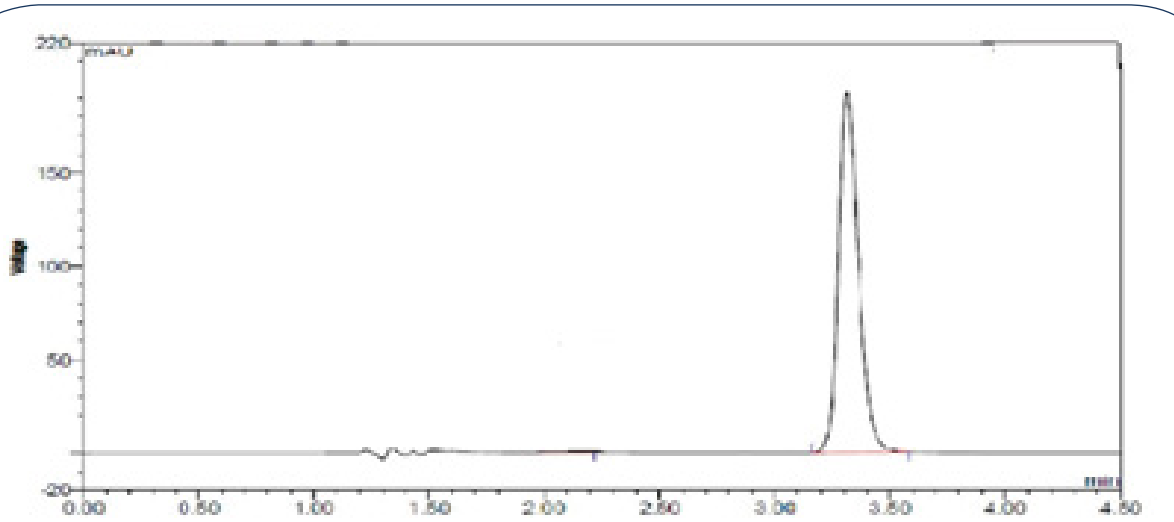

Figure 5. Chromatogram of standard aspirin (R_t=3.3 min)

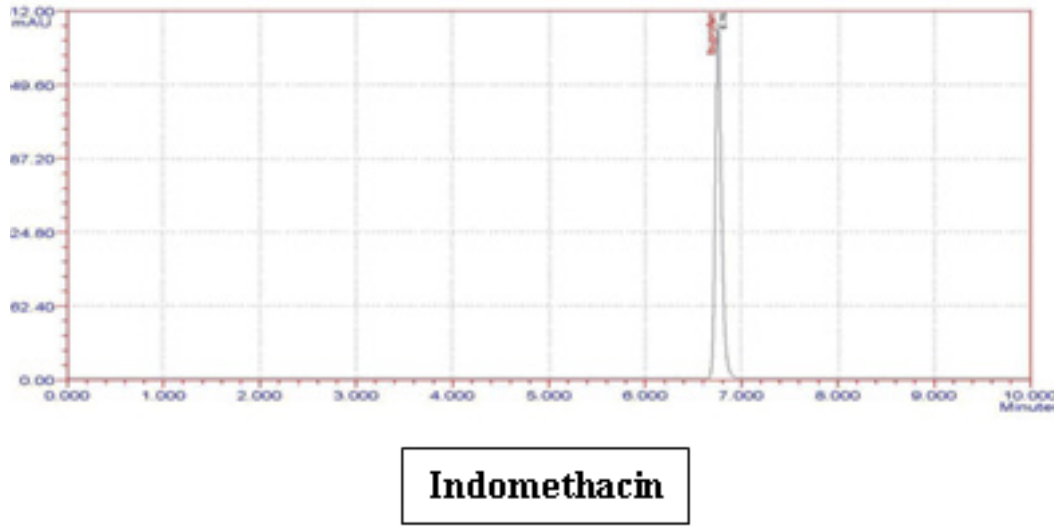

Figure 6. Chromatogram of standard indomethacin (R_t=6.8 min)

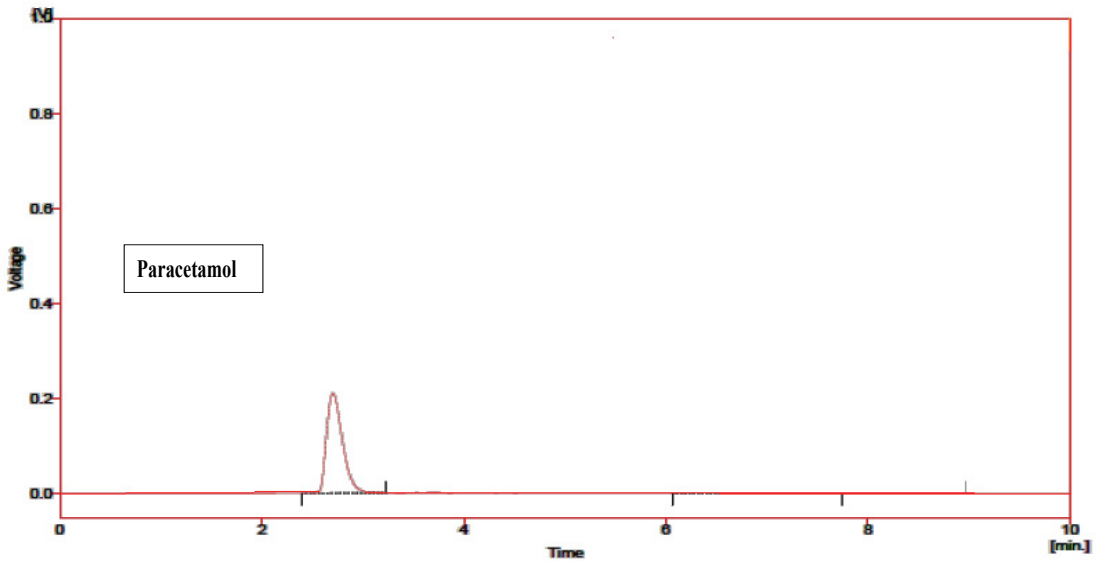

Figure 7. Chromatogram of standard paracetamol (R_t=2.5 min)

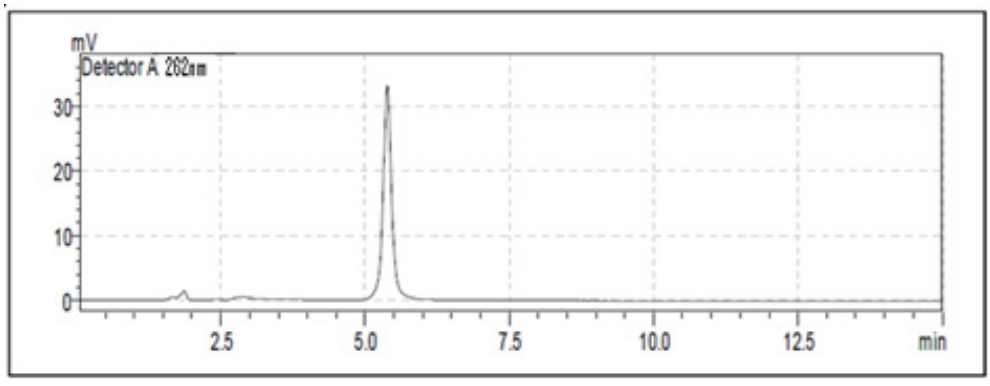

Figure 8. Chromatogram of Prodrug-A (10 µg/ml) (R_t=5.5 min)

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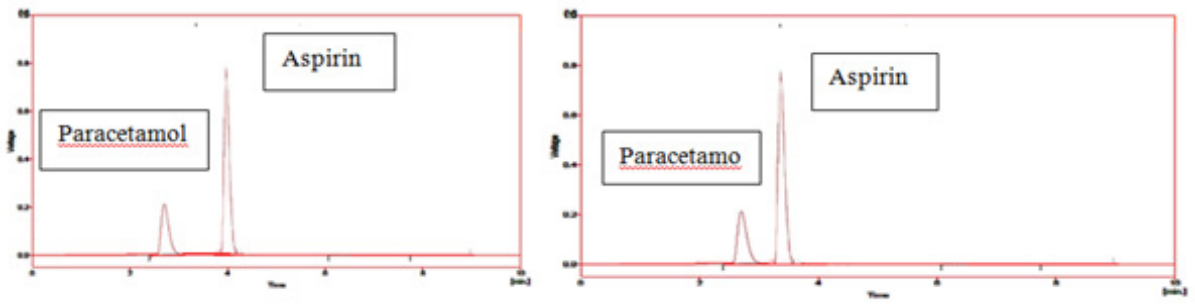

Figure 9. Chromatogram of Prodrug-A in acidic medium (HCl: pH=3.0) and in alkaline medium (NaOH: pH=7.5)

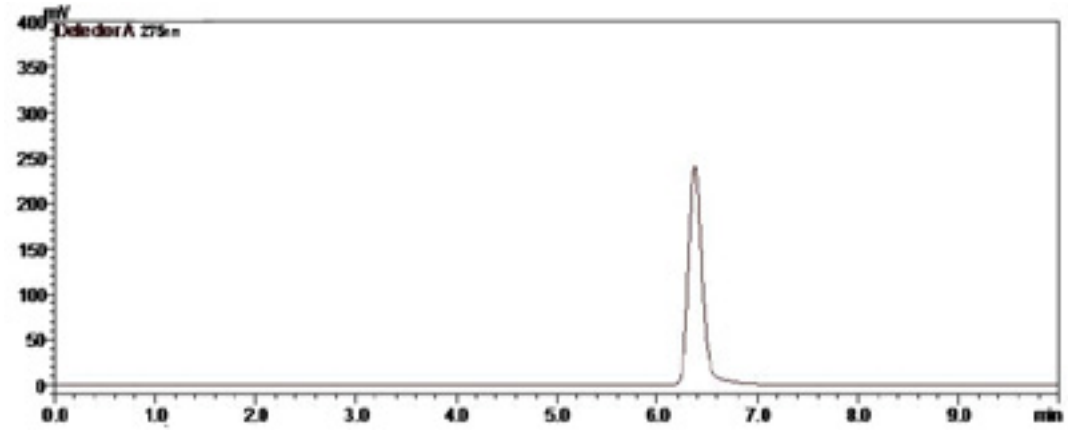

Figure 10. Chromatogram of Prodrug-B (100 µg/ml) (R_t=6.4 min)

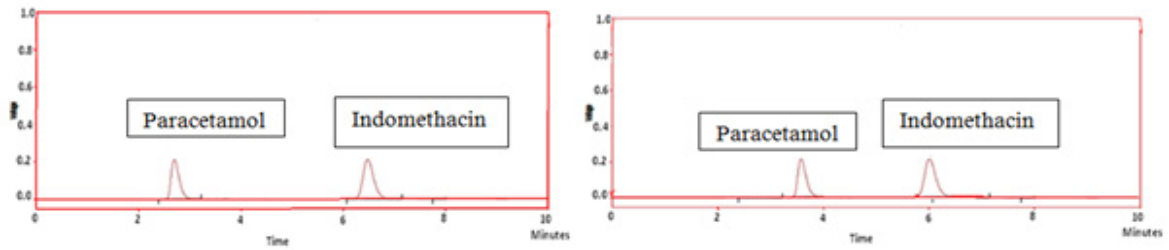

Figure 11. Chromatogram of Prodrug-B (indomethacin+paracetamol) in acidic medium (HCl: pH=3.5) and in alkaline medium (NaOH: pH=8.0)

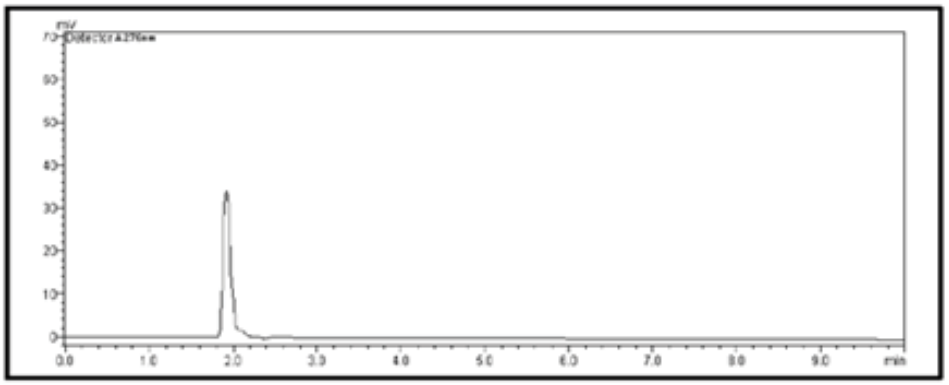

Figure 12. Chromatogram of codrug-A (30 µg/ml) (R_t=1.8 min)

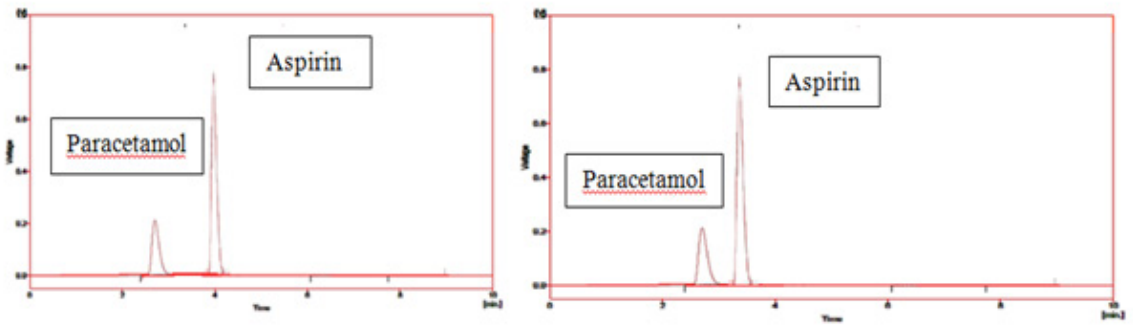

Figure 13. Chromatogram of Codrug-A in acidic medium (HCl: pH=3.0) and in alkaline medium (NaOH: pH=7.5)

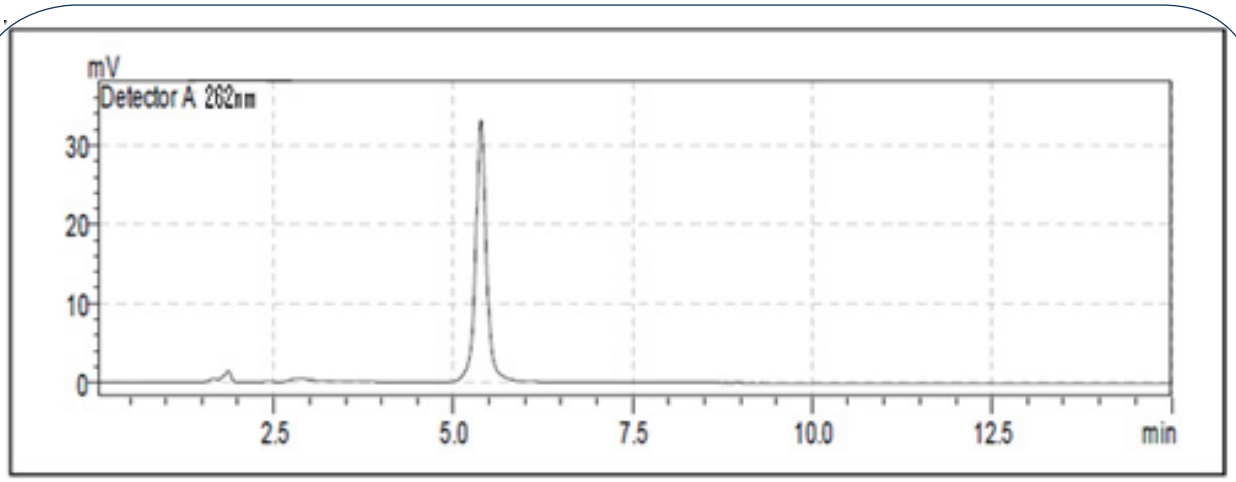

Figure 14. Chromatogram of Prodrug-B (10 µg/ml) (R_t=5.5 min)

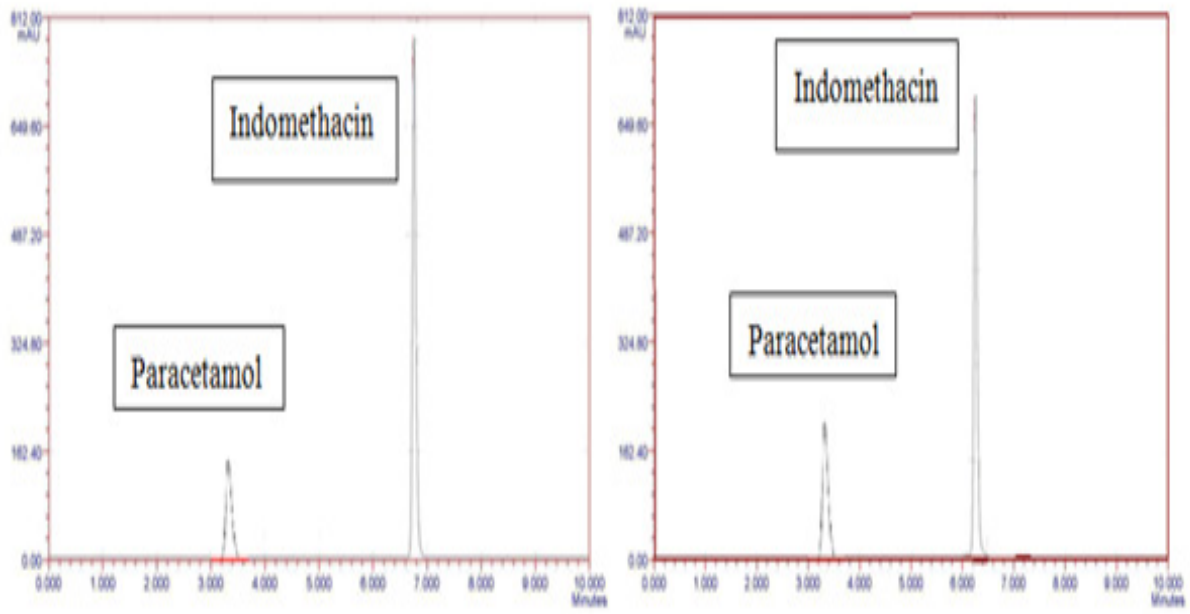

Figure 15. Chromatogram of Codrug-B in acidic medium (HCl: pH=3.0) and in alkaline medium (NaOH: pH=7.5)

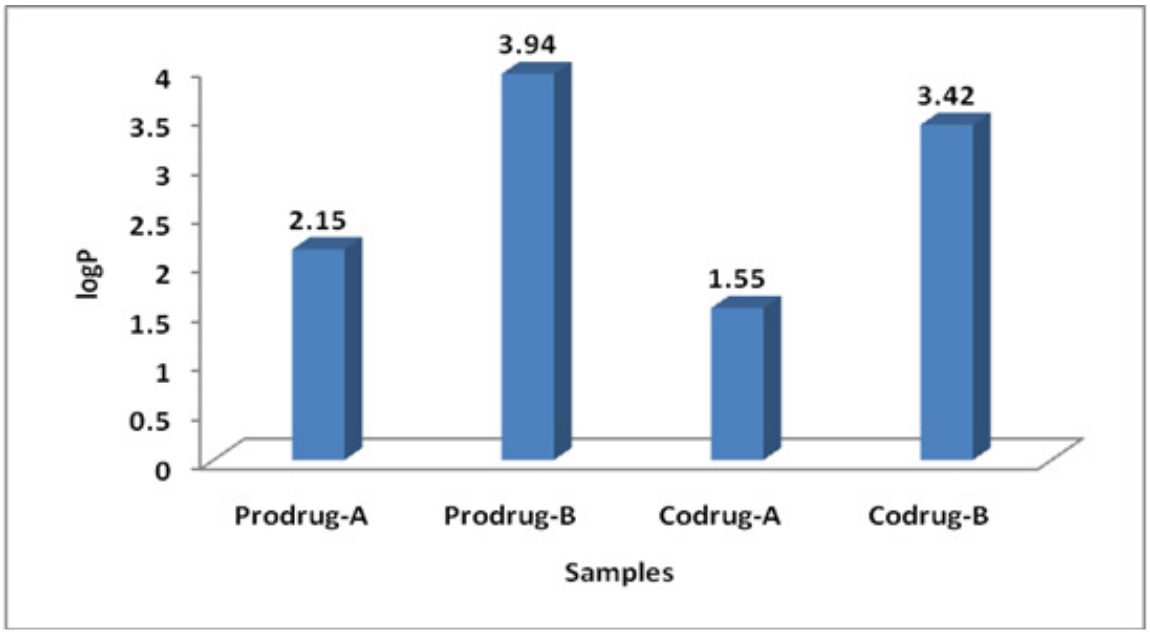

Figure 16. logP profiles of prodrug and codrugs

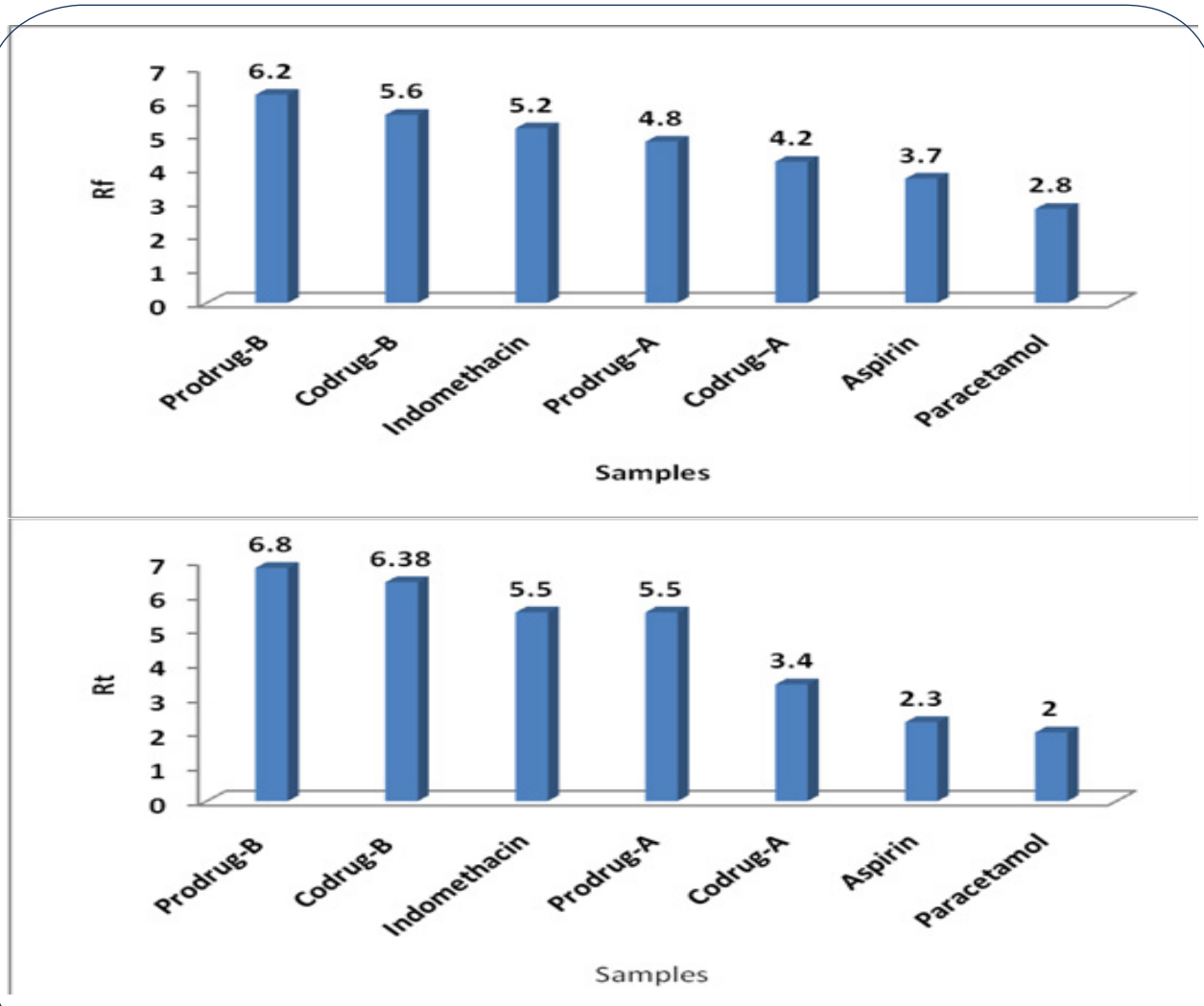

Figure 17. Comparative histogram of R_f (TLC) and R_t (HPLC) of samples