EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 10
345-352
Year: 2018

VARIATION OF RETENTION TIME BY HPLC STUDY OF COCRYSTALLIZED AS WELL AS MIXTURE OF ACTIVE PHARMACEUTICAL INGREDIENTS OF ASPIRIN, IBUPROFEN AND PARACETAMOL IN PHYSIOLOGICAL FLUIDS

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Article Received on 09/08/2018 **Article Revised on 29/08/2018** **Article Accepted on 19/09/2018**

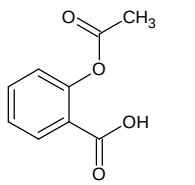
ABSTRACT

Aspirin, Ibuprofen, Indomethacin, Diclofenac sodium and Paracetamol are taken as NSAIDs. All drugs have free carboxylic acid group (–COOH) and paracetamol has free phenolic group (–OH) so all are acidic in nature. The combination of drugs is treated for HPLC as well as for codrug: Aspirin & Paracetamol, Ibuprofen & Paracetamol, Indomethacin & Paracetamol, Diclofenac & Paracetamol and Ibuprofen & Diclofenac. Aspirin & Paracetamol, Ibuprofen & Paracetamol, Indomethacin & Paracetamol, Diclofenac & Paracetamol and Ibuprofen & Diclofenac codrugs are formed by hydrogen bonding, ionic interactions, Van der Waals interactions and π–interactions. HPLC study was performed for all individual mixtures as well as codrugs of these and R_t values were recorded. It has been proved that R_t values of mixture are greater than R_t of cocrystallized products.

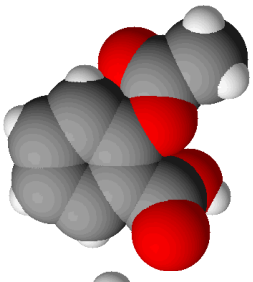
KEYWORDS: Aspirin, Ibuprofen, Indomethacin, Diclofenac sodium, Paracetamol, Mobile phase, TLC–R_f value, HPLC–R_t value, API, Mobile phase, Co–crystallization, Hydrogen bonding, Ionic interactions, Van der Waals interactions, π–interactions.

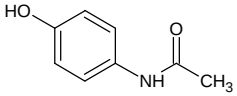
INTRODUCTION

Prodrug is a substance which after administration is metabolized into a pharmacologically active drug. Actually Prodrug has least medicinal value in *in–vitro/in–vivo* but after biotransformation by metabolism in *in–vivo* it releases the active medicament. A drug is a substance which is a chemical entity, has definite structural skeleton, obtained by natural or synthetic or semisynthetic source, which can fit on bioreceptor platform having controlling capacity to control over the biochemical malfunction. Every drug is xenobiotic because it is coming from outer source (xeno) and active in biological unit (biotic). 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 & biochemical parameters by pharmacodynamics.[1-3]

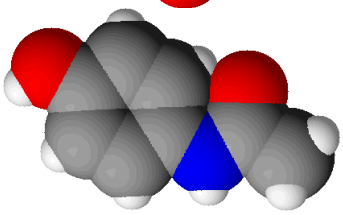


2-(acetyloxy)benzoic acid
Aspirin





N-(4-hydroxyphenyl)acetamide
Paracetamol



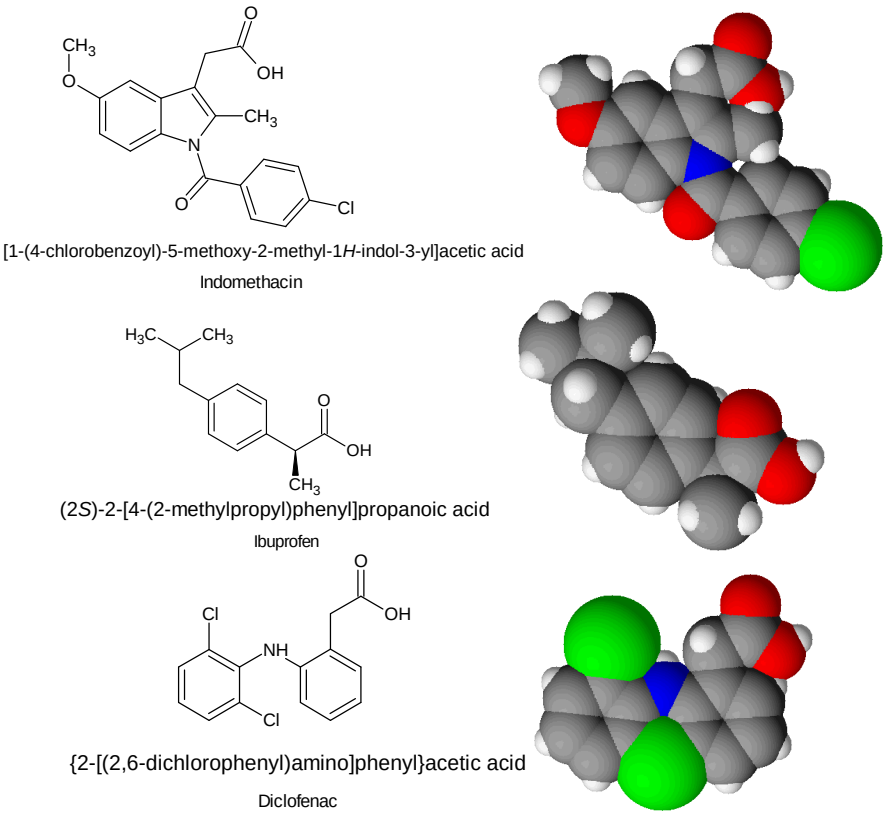


Figure 1: Active Pharmaceutical Ingredients.

Codrug or "mutual prodrug" consists of two synergistic drugs chemically linked together, in order to improve the drug delivery properties of one or both drugs. The constituent drugs are indicated for the same disease, but may exert different therapeutic effects via disparate mechanisms of action. There exists a disagreement on the meaning of the term "cocrystal." One definition states that a cocrystal is a crystalline structure composed of at least two components, where the components may be atoms, ions or molecules. This definition is sometimes extended to specify that the components be solid in their pure forms at ambient conditions. However, it has been argued that this separation based on ambient phase is arbitrary. A more inclusive definition is that cocrystals "consist of two or more components that form a unique crystalline structure having unique properties."

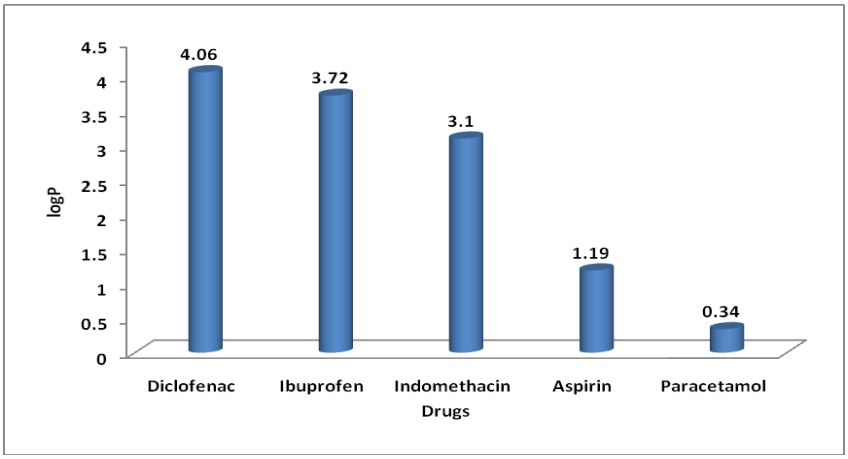


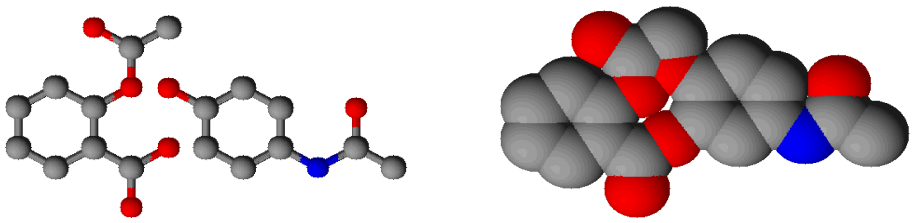
Figure-2: logP.

logP Explanation: Diclofenac (4.06)>Ibuprofen (3.72)>Indomethacin (3.1)>Aspirin (1.19)>Paracetamol (0.34).

Due to variation in the use of the term, structures such as solvates and clathrates may or may not be considered cocrystals in a given situation. It should be noted that the difference between a crystalline salt and a cocrystal lies merely in the transfer of a proton. The transfer of protons from one component to another in a crystal is dependent on the environment. For this reason, crystalline salts and cocrystals may be thought of as two ends of a proton transfer spectrum, where the salt has completed the proton transfer at one end and an absence of proton

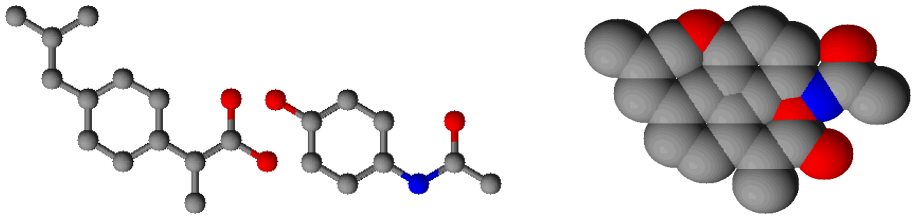
transfer exists for cocrystals at the other end. Cocrystal structures exhibit long–range order and the components interact via non–covalent interactions such as hydrogen bonding, ionic interactions, Van der Waals interactions and π–interactions. The intermolecular interactions and resulting crystal structures can generate physical and chemical properties that differ from the properties of the individual components. Such properties include melting point, solubility, chemical stability, and mechanical properties. Some cocrystals have been observed to exist as polymorphs, which may display different physical properties depending on the form of the crystal.[4-6]

Phase diagrams determined from the "contact method" of thermal microscopy proved valuable in the discovery of new cocrystals. The construction of these phase diagrams is made possible due to the change in melting point upon cocrystallization. Two crystalline substances are deposited on either side of a microscope slide and are sequentially melted and re–solidified. This process creates thin films of each substance with a contact zone in the middle. A melting point phase diagram may be constructed by slow heating of the slide under a microscope and observation of the melting points of the various portions of the slide. For a simple binary phase diagram, if one eutectic point is observed then the substances do not form a cocrystal. If two eutectic points are observed, then the composition between these two points corresponds to the cocrystal.[7]



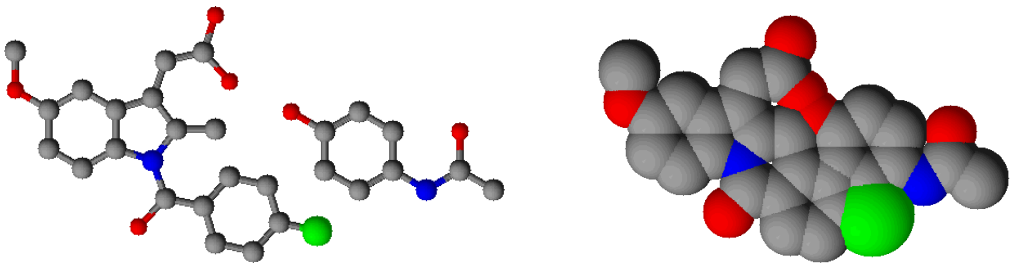
Explanation: Aspirin+Paracetamol codrug [showing 6 oxygen atoms (red) and 1 nitrogen atom (blue)=Total 7 hetero atoms].

Figure-3: Cocrystal of Aspirin & Paracetamol.



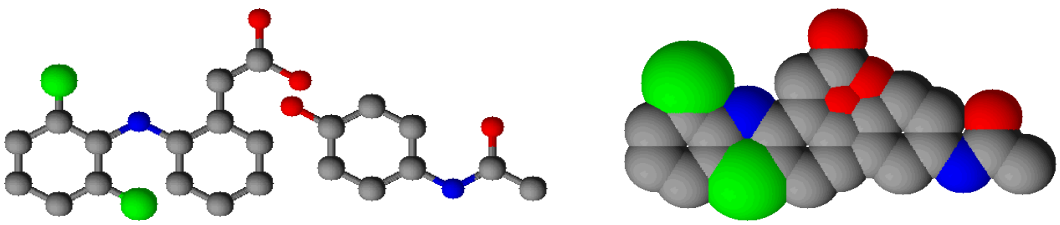
Explanation: Ibuprofen+Paracetamol [showing 4 oxygen atoms (red) and 1 nitrogen atom (blue)=Total 5 hetero atoms].

Figure 4: Cocrystal of Ibuprofen & Paracetamol.



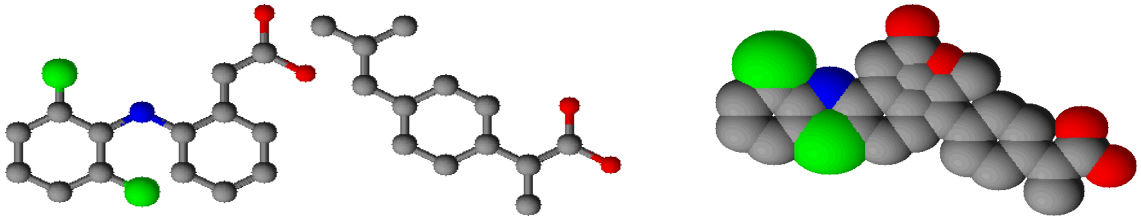
Explanation: Indomethacin+Paracetamol [showing 6 oxygen atom (red), 2 nitrogen atoms (blue) and 1 chlorine atom (green)=Total 9 hetero atoms].

Figure 5: Cocrystal of Indomethacin & Paracetamol.



Explanation: Diclofenac+Paracetamol [showing 4 oxygen atoms (red) and 2 nitrogen atoms (blue) and 2 chlorine atoms (green)=Total 8 hetero atoms].

Figure-6: Cocrystal of Diclofenac & Paracetamol.



Explanation: Ibuprofen+Diclofenac [showing 4 oxygen atoms (red) and 1 nitrogen atom (blue) and 2 chlorine atoms (green)=Total 7 hetero atoms].

Figure 7: Cocrystal of Ibuprofen & Diclofenac.

RESULT

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

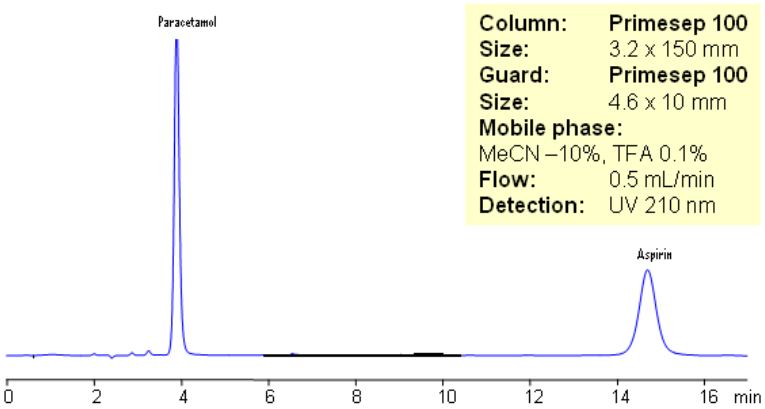
Experimental: Reagents and Materials, Prodrug-A synthesized in our college lab, Methanol (HPLC grade, Finar Chemicals Ltd, Ahmedabad, India), Water (HPLC grade, Finar Chemicals Ltd, Ahmedabad, India).

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).

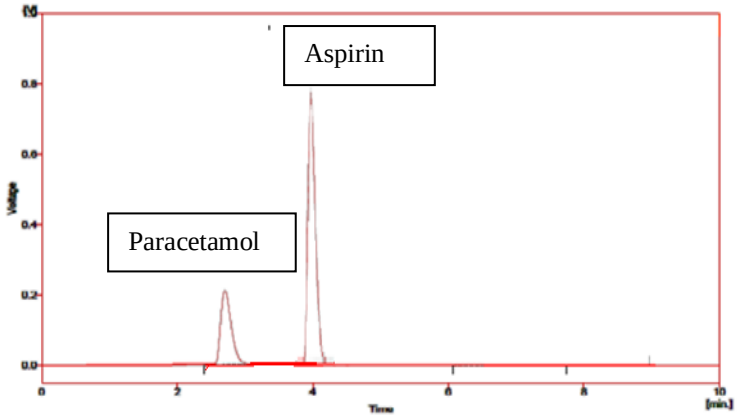
Table 1: Selection of mobile phase.

	Trials	Ratio	Remark		Trials	Ratio	Remark
Aspirin+ Paracetamol	1	Methanol: Water (60:40)	Tailing	***Ibuprofen+ Paracetamol***	1	ACN: Water (80:20)	Tailing
	2	ACN: Water (60:40)	Tailing		2	ACN: Methanol (80:20)	Tailing
	3	ACN: Water (70:30)	Tailing		3	ACN: Methanol (70:30)	Tailing
	4	Methanol: Water (60:40)	Tailing		4	Methanol: Water (80:20)	Tailing
	5	Methanol: Water (80:20)	**Symmetrical peak**		5	Methanol: Water (70:30)	**Symmetrical peak**
Indomethacin+ Paracetamol	1	Methanol: Water (50:50)	Tailing	***Diclofenac+ Paracetamol***	1	ACN: Water (70:30)	Tailing
	2	ACN: Water (60:40)	Tailing		2	ACN: Methanol (60:40)	Tailing
	3	ACN: Water (70:30)	Tailing		3	ACN: Methanol (50:50)	Tailing
	4	Methanol: Water (80:20)	Tailing		4	Methanol: Water (80:20)	Tailing
	5	Methanol: Water (80:20)	**Symmetrical peak**		5	Methanol: Water (70:30)	**Symmetrical peak**
Ibuprofen+ Diclofenac	1	Methanol: Water (60:40)	Tailing	*Explanation: TLC study has been done for all combinations and cocrystallized drugs and mixture to find out the exact mobile phase to fix the HPLC mobile phase. Various ratios of two solvents were studied by trial and error basis to select the exact mobile phase to run the spot of the drug properly.* ***Symmetrical peak*** *was obtained after* ***Tailing.*** ***R_f value of TLC focused over R_t value of HPLC.***			
	2	ACN: Water (60:40)	Tailing				
	3	ACN: Water (70:30)	Tailing				
	4	Methanol: Water (60:40)	Tailing				
	5	Methanol: Water (80:20)	**Symmetrical peak**				

1. *Aspirin+Paracetamol*

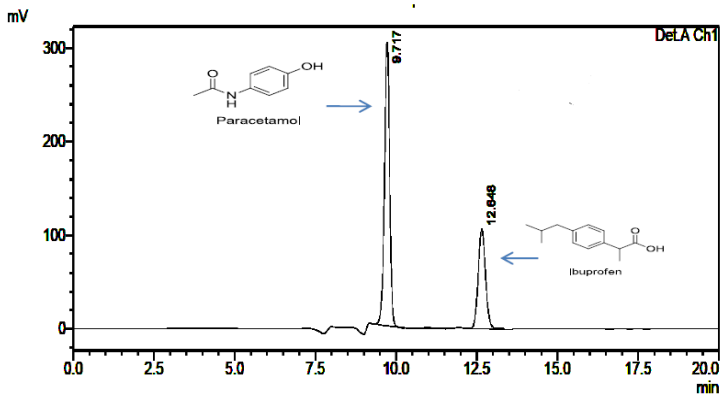


Explanation: Paracetamol shows R_t as 3.6min and aspirin as 14.8min in mixture.

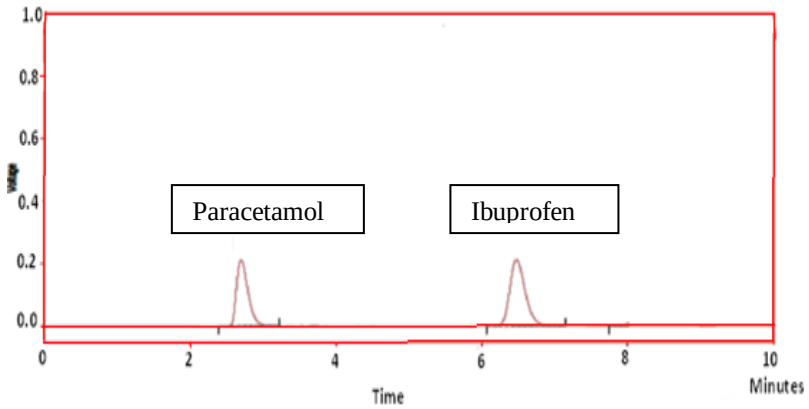


Explanation: Paracetamol has R_t=2.8min; Aspirin has R_t=4min.
Paracetamol shows R_t as 3.6min and aspirin as 14.8min in mixture and Paracetamol has R_t as 2.8min and Aspirin has R_t as 4min in cocrystallized drug.

2. *Ibuprofen+Paracetamol*

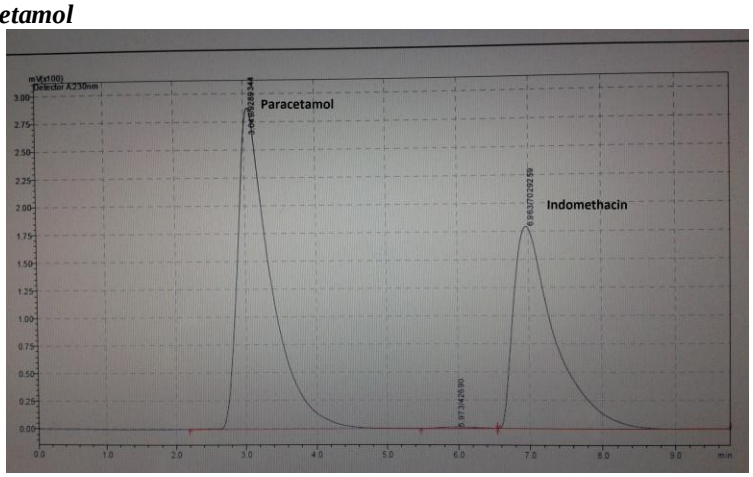


Explanation: Paracetamol shows R_t at 9.8min and ibuprofen as 12.5min.

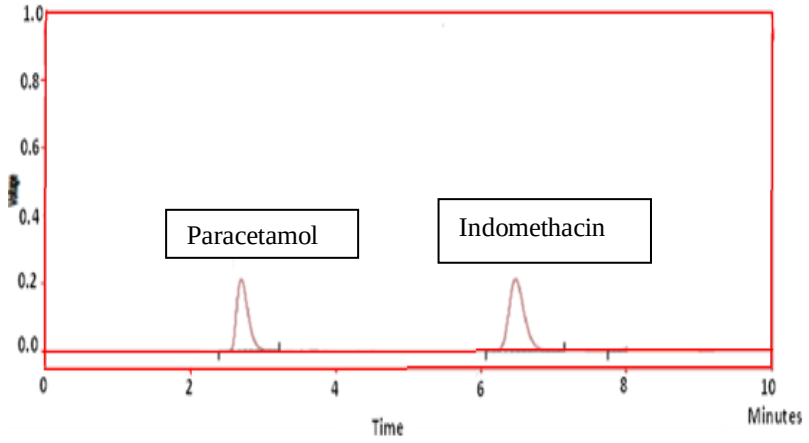


Explanation: Paracetamol has R_t=2.5min; Ibuprofen has R_t=6.35min.
Paracetamol shows R_t at 9.8min and ibuprofen as 12.5min in mixture and Paracetamol has R_t as 2.5min and Ibuprofen has R_t as 6.35min in cocrystallized drug.

3. *Indomethacin+Paracetamol*

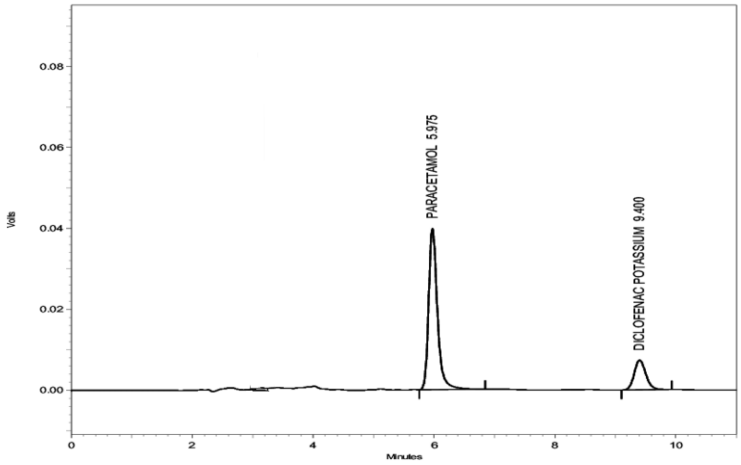


Explanation: Paracetamol shows R_t as 3.5min and indomethacin has 7min.

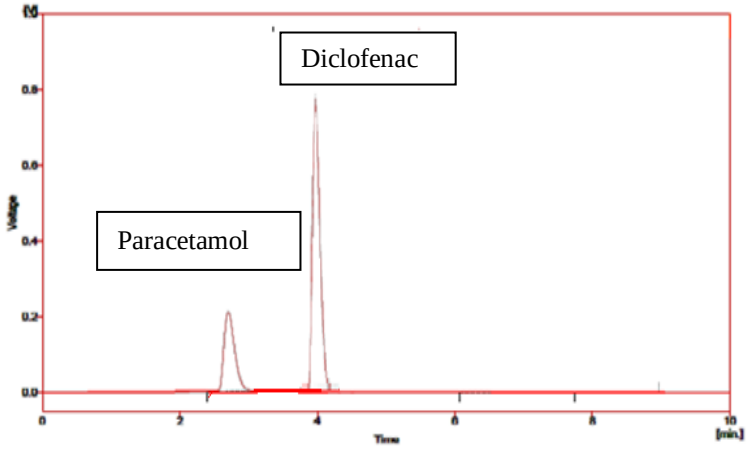


Explanation: Paracetamol has R_t=3.5min; Indomethacin has R_t= 6.15.
Paracetamol shows R_t as 3.5min and indomethacin has 7min in mixture and Paracetamol has R_t as 3.5min and Indomethacin has R_t as 6.15 in cocrystallized drug.

4. Diclofenac+Paracetamol

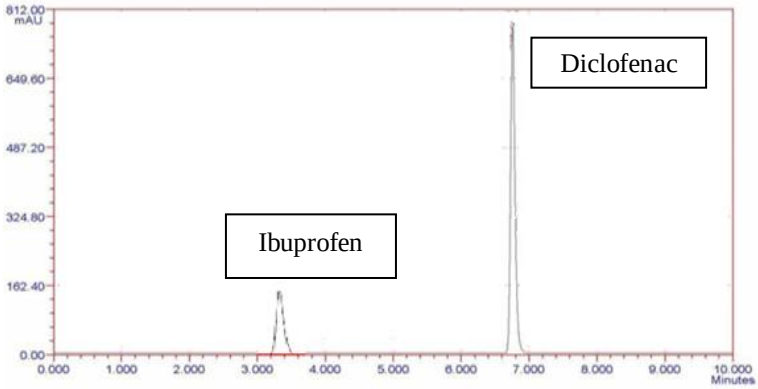


Explanation: Paracetamol shows R_t at 6min and diclofenac has 9.5min.

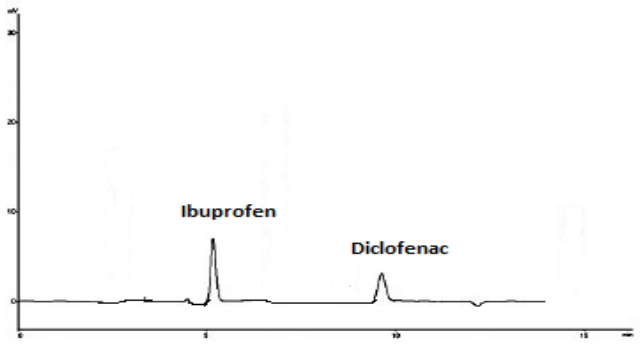


Explanation: Paracetamol has R_t=2.8min, Diclofenac has R_t=4min.
Paracetamol shows R_t at 6min and diclofenac has 9.5min in mixture and Paracetamol has R_t as 2.8min, Diclofenac has R_t as 4min in cocrystallized drug.

5. Ibuprofen+Diclofenac



Explanation: Ibuprofen has R_t=3.4min, diclofenac has R_t=6.8min.



Explanation: Ibuprofen shows R_t at 1.2min and Diclofenac has 2.6min.
Ibuprofen shows R_t at Ibuprofen has R_t at 3.4min, Diclofenac has R_t at 6.8min in mixture and Ibuprofen has R_t as 1.2min and Diclofenac has 2.6min in cocrystallized drug.

Figure 8: R_t values of mixtures and cocrystals

CONCLUSION

Cocrystallization makes the individual drugs affected by hydrogen bonding, ionic interactions, Van der Waals interactions and π–interactions so the physical nature of individual drugs change especially logP because partition coefficient makes any moiety to become soluble in polar and nonpolar solvent. Retention time (R_t) of HPLC shows the data that individual drug mixture takes much time when compared with cocrystal of the same. Hence it can be concluded that cocrystal forming by hydrogen bonding, ionic interactions, Van der Waals interactions and π–interactions all are physical property of a chemical substance that can easily change the logP to make it much water soluble so that the R_t of cocrystal HPLC is less that individual drug mixture. logP Explanation: Diclofenac (4.06) > Ibuprofen (3.72) > Indomethacin (3.1) > Aspirin (1.19) > Paracetamol (0.34). Diclofenac (4.06), Ibuprofen (3.72) and Indomethacin (3.1) have high logP (nonpolarity) when compared with Aspirin (1.19) and Paracetamol (0.34) so Diclofenac (4.06), Ibuprofen (3.72) and Indomethacin (3.1) when given with mixture it shows higher R_t rather than cocrystals. **R_t Mixture > R_t Cocrystals.** Hence the drugs made by cocrystallization releases faster than the same when in physical mixture form due to polymorphism. 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.

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