

HPLC STUDY OF ASPIRIN AND ASPIRIN DERIVATIVES

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ABSTRACT

A simple, rapid, precise and accurate HPLC method for differentiating aspirin from the derivatives of Aspirin synthesized. The instrument used for carrying out HPLC was Shimadzu LCVP2010C integrated system equipped with quaternary gradient pump, Column utilized Kromasil C₁₈ (180 X 4.6mm), 5 μ m, and the detection made possible using UV-Vis detector at a wavelength of 277nm and the mobile phase employed was acetonitrile: methanol 60:40(v/v). The R_t(Retention time) of aspirin was found to be 4.303 and that of derivatives as SR01- 2.543, SR02 -3.797, SR03-4.133, SR05-2.553, SR06-3.277, SR07-2.903. Proving the fact that the aspirin and its derivatives synthesized are pure.

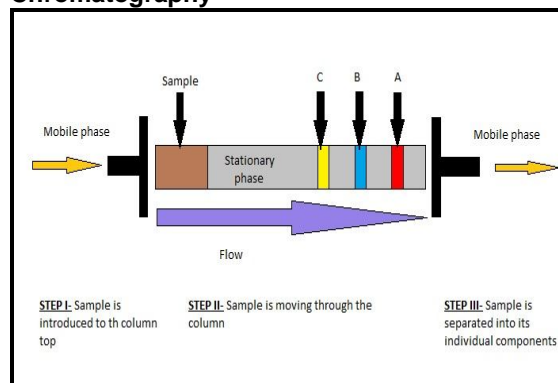
Keywords: Aspirin, HPLC, UV-Visible detector, Retention time.

INTRODUCTION

Chromatographic process can be defined as separation technique involving mass-transfer between stationary and mobile phase. HPLC utilizes a liquid mobile phase to separate the components of a mixture. The stationary phase can be a liquid or a solid phase. These components are first dissolved in a solvent, and then forced to flow through a chromatographic column under high pressure. In the column, the mixture separates into its components. The amount of resolution is important, and is dependent upon the extent of interaction between the solute components and the stationary phase. The stationary phase is defined as the immobile packing material in the column. The interaction of the solute with mobile and stationary phases can be manipulated through different choices of both solvents and stationary phases. As a result, HPLC acquires a high degree of versatility not found in other chromatographic systems and it has the ability to easily separate a wide variety of chemical mixtures.¹ HPLC is a dynamic adsorption process. Analyte molecules, while moving through the porous packing beads, tend to interact with the surface adsorption sites. Depending on the HPLC mode, the different types of the adsorption forces may be included in the retention process: Hydrophobic (non-specific) interactions are the main ones in reversed-

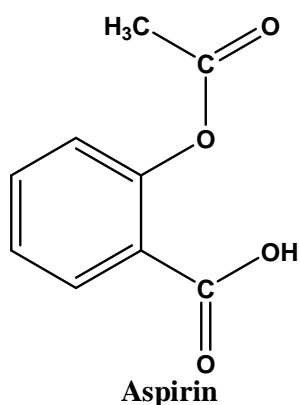
phase (RP) separations. Dipole-dipole (polar) interactions are dominant in normal phase (NP) mode. Ionic interactions are responsible for the retention in ion-exchange chromatography. All these interactions are competitive. Analyte molecules are competing with the eluent molecules for the adsorption sites. So, the stronger analyte molecules interact with the surface. The weaker the eluent interaction, the longer the analyte will be retained on the surface. SEC (size-exclusion chromatography) is another case. It is the separation of the mixture by the molecular size of its components, the bigger the molecule, lesser the chances for it to be retained.²

Simplified Principle of Column Liquid Chromatography³



In HPLC the mobile phase flow by using a high-pressure pump through a stationary phase composed of small particles of 2-5 micrometer size range. HPLC is the method of choice for the separation of non volatile organic compounds such as hydrocarbons steroids, pesticides, strongly polar and ionic compounds such as amino acids, nucleic acids high molecular weight compounds such as synthetic polymers and biopolymers such as polypeptides, proteins, polynucleotide's, carbohydrates and thermo labile and decomposable compounds such as terpenoids.⁴

DRUG PROFILE Chemical structure



Physical state – White crystalline powder which is weakly acidic
Molecular formula - C₉H₈O₄
Molecular mass – 180.157 g/mol
Melting point – 135°C
Boiling point – 140°C
To account for the purity of aspirin the prepared derivatives of aspirin were analysed using HPLC.

EXPERIMENTAL

Synthesis of Aspirin^{4,5,6,7}

Requirements

Salicylic acid- 10g, Pyridine- 7 ml, acetyl chloride- 7.5 ml.

Procedure

In a 100 ml conical flask which contained 7 ml of pyridine, 10g of salicylic acid was dissolved. Without delay it was made to run in 7.5 ml (8.3 g) of acetyl chloride, adding about 1 ml of the chloride at a time and the mixture was shaken continuously during the addition. The heat of the reaction would cause the temperature of the mixture to rise rapidly. Therefore the latter should be maintained between 50° and 60° C

throughout the addition. The flask was cooled occasionally in cold water whenever necessary. Finally the mixture was heated on a boiling water-bath for 5 minutes. After cooling in cold water it was poured as a thin stream into 300 ml of cold water and stirred the mixture vigorously. The solid product was filtered using a pump, washed thoroughly with water and drained. Recrystallisation from a mixture of equal volumes of water and acetic acid was done. The product was dried and recrystallised from benzene. The acetylsalicylic acid was obtained as colourless crystals³¹.

Test for Differentiation of Salicylic Acid and Aspirin.

Test	Observation	Aspirin
Ferric chloride Test	Buff coloured solution	Aspirin present
	Violet coloured solution	Salicylic acid

Synthesis of Derivatives of Aspirin^{8,9}

Requirements

Aspirin-

1.8g, Alcohol (Methanol/Ethanol/Isopropylalcohol/Phenol/Benzyl alcohol/Resorcinol)*- 15ml, Thionyl chloride- 5ml, Ethyl acetate- 25ml, Sodium bicarbonate- 25ml, Distilled water- 30ml.

Procedure

To a stirred solution of aspirin (10mmol= (Molecular weight/100) g) in 15ml of alcohol, under ice cooling, thionyl chloride was added dropwise over 10 minutes. After the reaction mixture was stirred for 3 hours alcohol was distilled out and 25ml of water was added. The separated ester was extracted with ethyl acetate and washed with 25ml of saturated sodium bicarbonate solution. Drying with sodium sulphate and evaporation of ethyl acetate gave the derivative in pure form. Crystalline products were filtered and dried. Liquid products were separated into their respective aqueous and organic layers.

HPLC

Instrument used: Shimadzu LCVP2010C integrated system equipped with quaternary gradient pump,
Column used: Kromasil C₁₈ (180 X 4.6mm), 5µm
Detector used: UV-Vis detector
Wavelength: 277nm
Mobile Phase: Acetonitrile: Methanol 60:40(v/v)

RESULTS AND DISCUSSION

Table 1: HPLC data of synthesized compounds

Compound Code	Retention time (R _t)
Aspirin	4.303
SR-01	2.543
SR-02	3.797
SR-03	4.133
SR-05	2.553
SR-06	3.277
SR-07	2.903

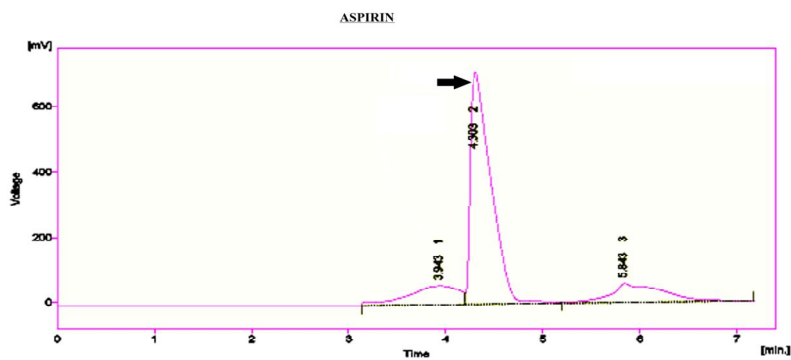


Fig. 1: Aspirin

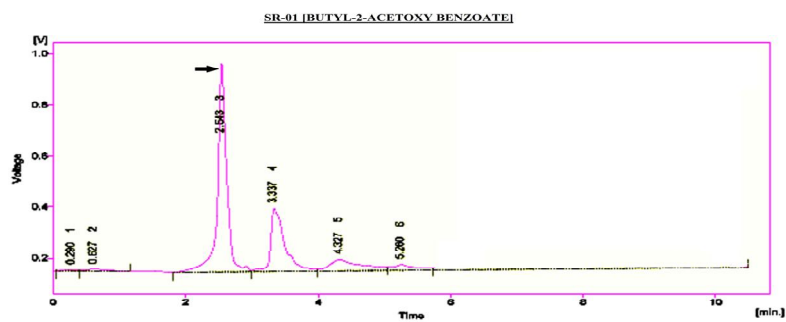


Fig. 2: SR-01(butyl-2-acetoxy benzoate)

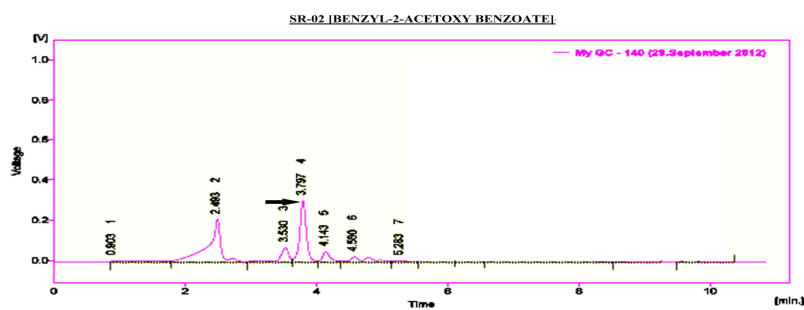


Fig. 3: SR-02(benzyl-2-acetoxy benzoate)

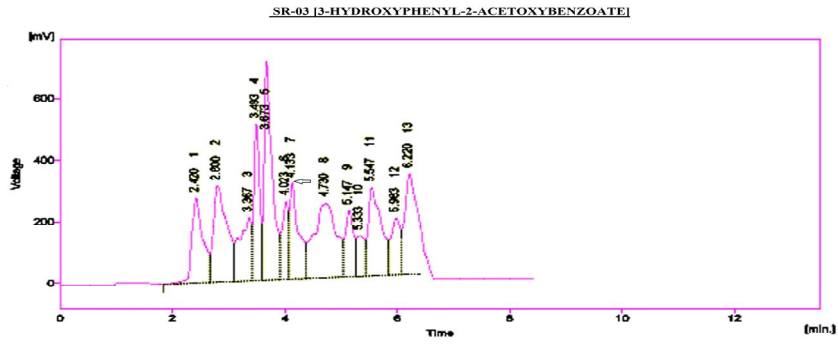


Fig. 4: SR-03(3- hydroxy phenyl-2-acetoxy benzoate)

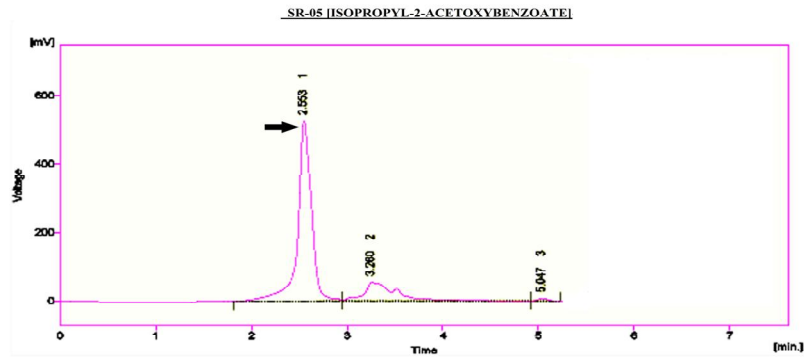


Fig. 5: SR-05(isopropyl-2-acetoxy benzoate)

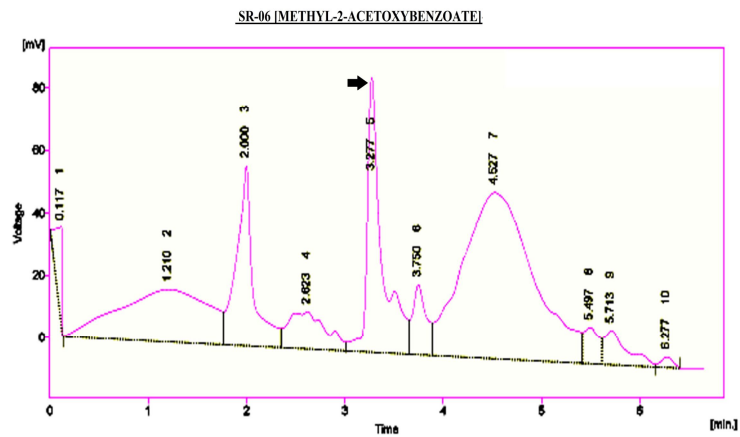


Fig. 6: SR-06(methyl-2-acetoxy benzoate)

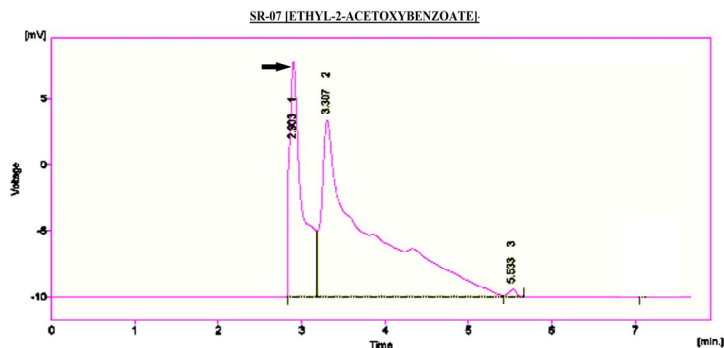


Fig. 7: SR-07(ethyl-2-acetoxy benzoate)

RESULTS AND DISCUSSION

The R_t (Retention time) of aspirin was found to be 4.303 and that of derivatives as SR01-2.543, SR02 -3.797, SR03-4.133, SR05-2.553, SR06-3.277, SR07-2.903. Proving the fact that the aspirin and its derivatives synthesized are pure.

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