

Preparation and in Vitro Evaluation of Tablets Containing Triple Drug Combination Used for Prevention/treatment of Cardiovascular Diseases

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Abstract: Patients suffering from cardiovascular disease are usually treated with concomitant administration of cholesterol lowering drugs, aspirin and beta blockers. Therefore, the objective of this project is to formulate and evaluate a triple drug combination tablet containing aspirin, simvastatin and propranolol HCl. The interaction of aspirin with both chosen drugs was studied by using Fourier-transformer infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC). Binary physical mixtures of aspirin with either simvastatin or propranolol HCl were prepared, in different weight ratios, as well as their ternary mixture. FT-IR spectra showed no molecular interaction between aspirin and either simvastatin or propranolol HCl in their physical mixtures. The DSC traces depicted lowering of melting points of aspirin and simvastatin by the action of each one on the other during heating. A single oral tablet containing three drugs was formulated and evaluated. Quantitative detection of the three individual drugs in presence of each other in the prepared tablet was determined by using HPLC method. It was found that HPLC is sensitive, reproducible and valid for determination of aspirin, simvastatin and propranolol HCl in the tablet form. The data revealed promising formula for improved relief patient compliance during treatment or prevention of cardiovascular diseases.

Key words: Aspirin, simvastatin, propranolol HCl, FT-IR, DSC, Dissolution.

INTRODUCTION

Pharmaceuticals preparations containing multiple active ingredients are essentially utilized to assure patient compliance. The treatment of many diseases usually requires the oral administration of more than one drug seeking a good treatment. The regimen of mutual administration of drugs in separate dosage forms may lead to many unrequired inferior treatment. This may be due to the un-necessary administration of large amounts of excipients, drug-excipient interaction, drug-drug interaction in addition to low patient compliances. For example, patients suffering from cardiovascular disease (ischaemic heart disease) usually are treated with concomitant administration of statins, aspirin and beta blockers. It was reported that combination of those three drugs reduced mortality by 83 % in high risk patients with ischaemic heart disease (Hippisley-Cox and Coupland 2005). Numerous clinical trials have demonstrated the value of long term management with ACE inhibitors, beta-blockers, statins, and aspirin (ASA) in reducing the risk of cardiovascular events and mortality in patients after acute coronary syndromes (ACS) (Braunwald ., 2002, Antman *et al.* 2004). Some combinations of these drug classes have also showed reduced mortality (Herlitz *et al.*, 2001, Hognestad *et al.*, 2004, Krause *et al.*, 2004). Simvastatin is a cholesterol-lowering medication that blocks the production of cholesterol in the body. It reduces low-density lipoprotein (LDL) cholesterol and total cholesterol in the blood (Jun *et al.*, 2007). Lowering cholesterol can help in preventing heart disease and hardening of the arteries (atherosclerosis), conditions that can lead to heart attack, stroke, and vascular disease. Statins inhibit an enzyme, HMG-CoA reductase, that controls the rate of cholesterol production in the body. The large reduction in total and LDL-cholesterol by using these drugs resulted in large reductions in heart attacks and heart disease-related deaths. Aspirin has been shown to reduce the risk of future heart attacks in patients who have already had one. Aspirin seems to work by reducing the stickiness of the platelets so that blood clots do not form as readily. Aspirin produces irreversible antiplatelet effect that can prevent primary and secondary coronary artery disease. Aspirin also reduces chances of ischemic complications after percutaneous coronary intervention (Peto *et al.*, 1988,

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Hennekens *et al* 1989, Manson *et al.*, 1991, Fuster *et al.*, 1993). Beta-blockers (e.g. propranolol HCl) are used to treat high blood pressure (hypertension), abnormal heart rhythms (arrhythmias), and chest pain (angina). The beta blockers reduced cardiovascular death and reinfarction in patients with history of myocardial infarction (Frishman and Cheng, 1999). Propranolol HCl slows the heart rate and makes it beat with less contracting force so blood pressure drops and the heart works less hard. The amount of risk reduction shown by aspirin and beta-blocker is similar to that of cholesterol lowering, making all three as important in the treatment of heart diseases. Therefore, combining aspirin, simvastatin and propranolol HCl is thus recommended for secondary prevention of coronary heart disease (CHD). Thus, the combination of two or three used drugs in one dosage form may be beneficial in getting improved treatment with high performance of the dosage form.

The objective of this project is to formulate and evaluate a triple drug combination tablet containing aspirin (81mg), simvastatin (10mg) and propranolol HCl (10mg) to be used for prophylactic/ treatment of cardiovascular diseases. Therefore, physical and chemical compatibility of the selected three drugs in the dosage form are studied by FT-IR, DSC and HPLC methods. In addition, in-vitro evaluation of the formulated tablets including hardness, friability, disintegration, and dissolution was performed.

Experimental:

Materials:

Simvastatin and propranolol hydrochloride were gifted from Riyadh Pharma, CO., Riyadh, Saudi Arabia. Aspirin was purchased from E. Merck, D-6100, Darmstadt, Germany. Phosphoric acid, potassium dihydrogen phosphate, hydrochloric acid were purchased from Sigma chemical Co., St. Louis, Mo., U.S.A. Acetonitrile was purchased from BDH Chemicals Ltd., Poole, England. Avicel ph 102 was purchased from Winlab, UK. Hydroxypropyl β -cyclodextrin (HP- β -CD) was purchased from Fluka Chemikam GMBH, Switzerland. Sodium starch glycolate, and magnesium stearate were purchased from Riedel-DeHuen AG, Seeleze, Hannover. All solvents used for chromatographic determinations were of HPLC grade. All other reagents and solvents were of analytical grade.

Preparation of Physical Mixtures of Aspirin with Simvastatin and Propranolol HCl:

Ternary physical mixtures of aspirin with both simvastatin and propranolol HCl were prepared by ordered mixing using mortar and pestle. Binary physical mixtures of aspirin with either simvastatin or propranolol HCl were prepared in different weight ratios by the same above method. These samples were investigated by using FT-IR and DSC methods.

Compatibility Assuring Test Using Fourier Transformer Infrared (FT-IR) Spectroscopy:

FT-IR spectrophotometer (Nicolet 380 FT-IR, Thermo Scientific, USA) was used to obtain the spectra of the aspirin, simvastatin, propranolol HCl, and their physical mixtures by KBr disc method. Polystyrene disc was used as scanning reference before measurement of samples by FT-IR spectrophotometer in the range of wave number 4000-400 cm^{-1} . The sample mixtures were prepared in a smooth agate mortar and compressed into a disc of 13-mm diameter using hydraulic press.

Compatibility Assuring Test Using Differential Scanning Calorimetry (DSC):

DSC measurement of each drug and their physical mixtures was carried out using Shimadzu DSC-60 with TA-60WS (Japan) equipped with software computer program. Running was operated under nitrogen purge gas with the rate 40-50 ml/min, and heating rate of 10 $^{\circ}\text{C}/\text{min}$. The weights of the samples were in the range of 2-5 mg. The temperature range of DSC runs was from 25 $^{\circ}\text{C}$ to 200 $^{\circ}\text{C}$. The DSC thermograms were recorded for samples sealed in aluminum pans and indium was used as standard for calibrating the instrument. The peak temperature of melting of each sample and the heat of fusion were determined from DSC traces by thermal analysis program. Phase diagram of aspirin with simvastatin was constructed from their DSC data of binary mixtures in various weight ratios.

Formulation of Triple Tablets:

Triple tablets containing aspirin (81mg), simvastatin (10mg) and propranolol HCL (10mg) were prepared by the direct compression technique. Components of each formula are shown in Table 1 were mixed in turbula mixer (type S27, Erweka, Apparatebau, Germany) and then directly compressed into tablets using a single punch tablet machine (type EKO, Erweka, Apparatebau, Germany) using 8 mm concave punches. Tablets' crushing strength was kept within the range of 4-8 kp and tablet weight to be around 280 mg. Avicel is used as diluent, magnesium stearate as lubricant, sodium starch glycolate as disintegrant. HP β -CD is used to improve

the dissolution of simvastatin because the dissolution rate of simvastatin alone in powder form in 0.1 N HCl gave very slow dissolution after two hours.

Table 1: Composition of the triple therapy tablet formula

Component	Amounts (mg)
Aspirin	81
Simvastatin	40 ^a
Prpranolol Hcl	10
Avicel PH 102	130
Magnesium stearate	2
Sodium starch glycolate	17
Total weight	280

a; 40mg solid dispersion with HP β -CD is equivalent to 10 mg simvastatin.

Preparation of Simvastatin-HP β -CD Solid Dispersion:

Solid dispersion of simvastatin with HP β -CD in 1:3 weight ratio was prepared by solvent co-evaporation method. Accurate weights of simvastatin and HP β -CD were dissolved in accurate volume of ethanol, and then the solvent was evaporated at room temperature. Weights of this solid dispersion equivalent to 10mg simvastatin were used in preparation of the triple therapy tablets.

Evaluation of the Prepared Triple Tablets:

The obtained tablets were evaluated with regard to uniformity of weight, disintegration time, and friability according to B.P. 2005. Friability of 10 tablets were measured using friability tester (type TA3R, Erweka, Apparatebau, Germany).

Hardness of tablets was measured using hardness tester (type TBH28, Erweka, Apparatebau, Germany) and the mean was taken. Thickness of tablets was also determined by micrometer.

Dissolution Studies:

Dissolution study of triple tablets containing aspirin, simvastatin, and propranolol HCl was performed using USP dissolution apparatus 1 (Caleva Ltd., Model 85T), at 50 rpm in dissolution medium of 750 ml of 0.1N HCl. The temperature was maintained at 37 \pm 0.5 °C. At specified time intervals, 5 ml was withdrawn and substituted by freshly prepared dissolution medium thermostated at the same temperature. The amount dissolved of each component in tablets was determined by HPLC method. Dissolution of aspirin alone and simvastatin alone was carried out in 0.1 N HCl for comparison. Each release was run in triplicate.

HPLC Analysis Method:

A waters isocratic liquid chromatography system (Waters, Boston, Mass., USA) consisting of Waters 717 plus autosampler, Waters 1525 binary HPLC pump, Waters 2487 dual λ absorbance detector. All analyses were conducted at ambient temperature. Separations were performed on symmetry C18, 5 μ m, 4.6 X 150 mm waters column. The mobile phase used for separating simvasatin composed of acetonitrile and water in a ratio of 2:1 adjusted to pH 3 by phosphoric acid, and that used for separating aspirin consisting of acetonitrile and water in a ratio of 1:2 adjusted to pH 3 by phosphoric acid. The mobile phase used for separating propranolol hydrochloride was composed of 10 mM phosphate buffer and acetonitrile in a ratio of 680:320 adjusted to pH 3.5 by phosphoric acid. The mobile phases were membrane filtered (Millipore, 0.45 μ m pore size) and degassed using Nexul ultrasonic, Kodo technical Co., South Korea, then pumped at 1 ml/min. The eluent was monitored at 251 nm for detecting simvastatin, 230 nm for detecting aspirin and 230 nm for detecting propranolol HCl. Calibration curves, based on the average of peak height of various concentrations for each drug, were employed to evaluate the drug content or amount dissolved from the tablets. Standard solutions were assayed and calibration curves were constructed befor analysis of each sample to ensure reproducibility of the HPLC method.

RESULTS AND DISCUSSION

FT-IR spectra of aspirin alone, simvastatin alone, propranolol HCl and their ternary mixture were measured. Fig.1 shows FT-IR spectra of aspirin alone and its ternary mixture with simvastatin and propranolol HCl in 7:2:1 weight ratio. FT-IR spectrum of aspirin alone dedicated its characteristic peaks, from which the stretching vibration of two carbonyl bands at 1754 and 1689.35 cm^{-1} of acetyl and carboxylic groups, respectively, and stretching vibration of -CH- of benzene ring at 1605.5 cm^{-1} . The above characteristic peaks

of aspirin are not affected by presence of simvastatin and propranolol HCl in its ternary mixture and they appeared at 1753.74, 1693.4 and 1605.6 cm^{-1} . These results revealed that there is no molecular interaction between aspirin and either simvastatin or propranolol HCl in the solid state. For more confirmation of no molecular interaction between the three drugs, FT-IR spectra of binary mixture of aspirin and simvastatin in 8:2 and 5:5 weight ratios also showed no frequency shift of carbonyl groups of aspirin (data not shown). Conclusively, the chosen three drugs are compatible when mixed together in the solid state.

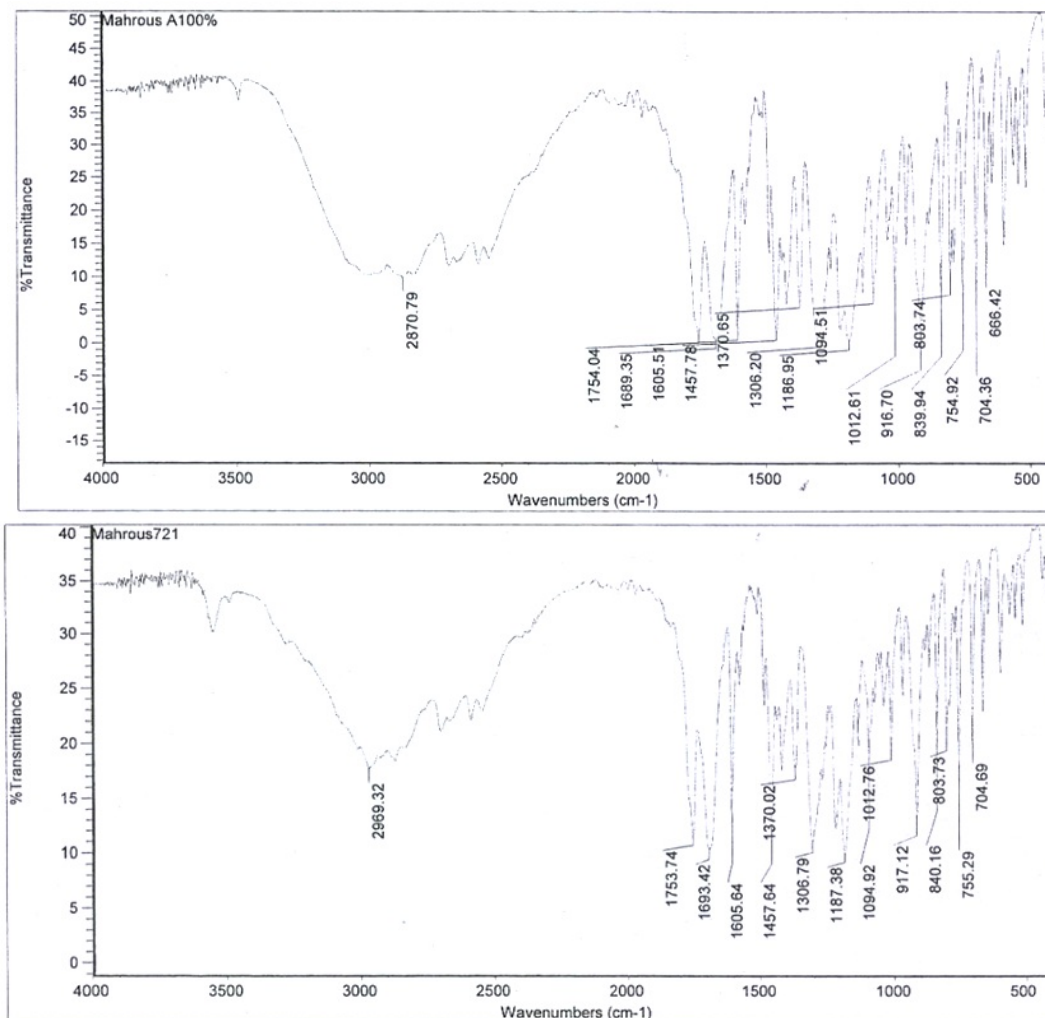


Fig.1: FT-IR spectra of aspirin (top) and its ternary mixture with both simvastatin and propranolol HCl in weight ratio of 7:2:1, respectively.

Fig. 2 depicts DSC curves of each drug alone and their ternary mixture. DSC traces of the studied drugs, gave endothermic peaks at 141.97, 140.9 and 163.74 $^{\circ}\text{C}$, which are due to the melting of aspirin, simvastatin and propranolol HCl, respectively. On the other hand, DSC curve of ternary mixture showed three endothermic peaks at 106.12, 133.36 and 162-182 $^{\circ}\text{C}$ (broad one). The last broad endothermic peak can be attributed to the fusion of propranolol HCl. To explain those two lowered endothermic peaks which appeared at 106.12 and 133.36 $^{\circ}\text{C}$, DSC of binary mixtures of aspirin and simvastatin in different weight ratios were recorded. Fig.3 shows DSC curves of aspirin alone and its binary mixtures with simvastatin in 9:1 and 8:2 weight ratios. Two distinct endothermic peaks at 106.91 (small) and 138.75 $^{\circ}\text{C}$ (large) for the binary mixture of 9:1 ratio, and at 108.46 and 136.38 $^{\circ}\text{C}$ for that of 8:2 ratio, were observed on the their DSC thermograms. From these data of DSC, the lowered two endothermic peaks appeared in case of ternary mixture are nearly similar to those appeared in case of binary mixture of aspirin with simvastatin. This lowering of melting points may be due to the effect of each drug on the other at higher temperature above 100 $^{\circ}\text{C}$ during DSC running.

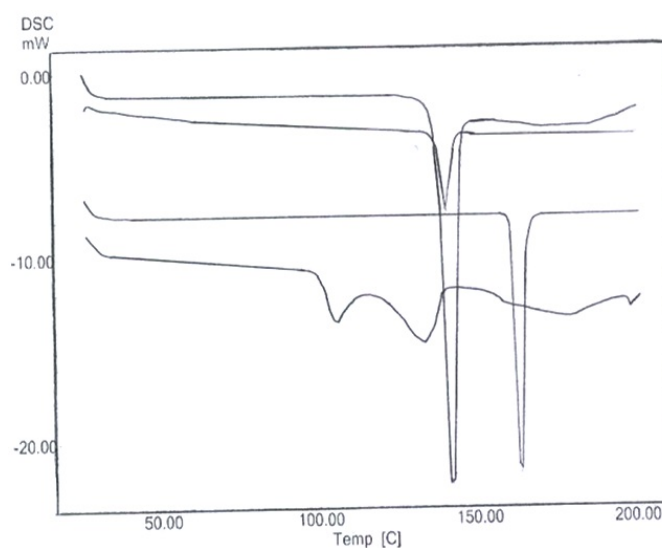


Fig. 2: DSC curves of aspirin, simvastatin, propranolol HCl and their ternary mixture in weight ratio of 7:2:1, from top to bottom, respectively.

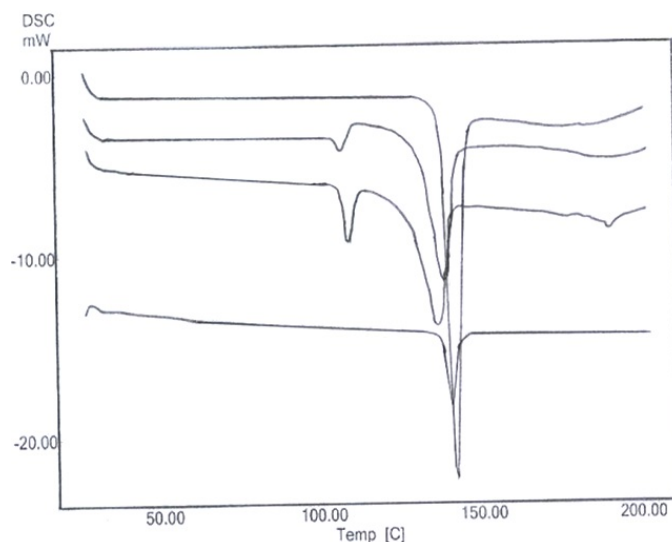


Fig. 3: DSC curves of binary physical mixtures of aspirin with simvastatin in weight ratios; 10:0, 9:1, 8:2, and 0:10, from top to bottom, respectively.

Fig.4 shows the phase diagram of aspirin with simvastatin constructed from their DSC data. The peak temperatures of melting endotherm of both drugs were lowered without formation of eutectic point which indicates that no interactions were detected between the studied drugs. Similar results were reported in which no interactions were detected between aspirin and phenobarbital although their melting points were lowered in their binary mixtures (Guillory *et al.*, 1969). It was also reported that paracetamol forms eutectic mixtures with aspirin and propyphenazone (Zolac *et al.*, 1999), and cloperastine hydrochloride (Sakata *et al.*, 2007), which is accompanied with lowering their melting points. Another report examined the potential of combining nifedipine and atenolol in a single tablet, lowering of their melting points was detected on DSC traces of their binary mixtures (Iglesias *et al.*, 1998). Generally, DSC and FT-IR have been frequently used to investigate compatibility or interaction between medicinal ingredients, and between drugs and excipients in solid dosage forms (Ahmed, 2000, Saleh *et al.*, 2003, Oberoi, *et al.*, 2005).

After these pre-formulation studies, tablets containing triple drug combination of aspirin, simvastatin, and propranolol HCl were prepared and evaluated according to B.P. It was found that the prepared tablets gave disintegration time of 6 minutes, and friability of loss 0.4 % which they comply with BP limits. Suitable

hardness value of 5.7 Kp of the prepared tablets was calculated, indicating good mechanical properties of tablets. Thickness of tablets was also measured and found to be 4.4 mm. The prepared tablets complied with BP limits of drug content.

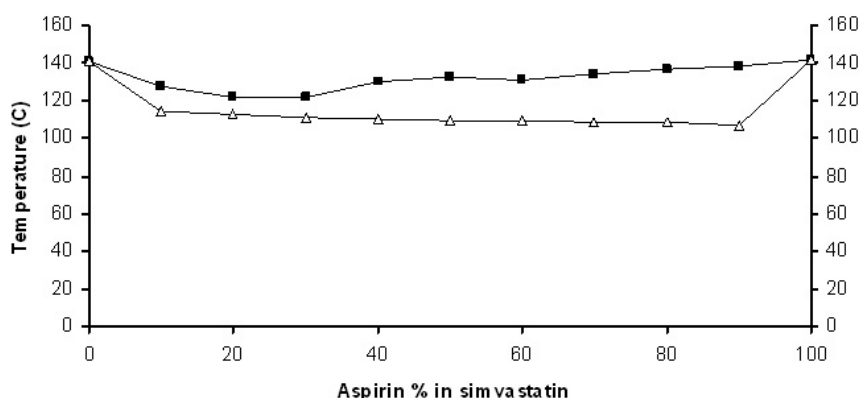


Fig. 4: Phase diagram for the aspirin-simvastatin system constructed from DSC data.

Fig. 5 shows the typical high-performance liquid chromatograms of propranolol HCl, aspirin and simvastatin from left to right. The RSD% (relative standard deviation) of used HPLC method ranged from 0.8 to 11.5 through the intraday measurements. This confirmed the reproducibility, accuracy and precision of the analytical method used during this study.

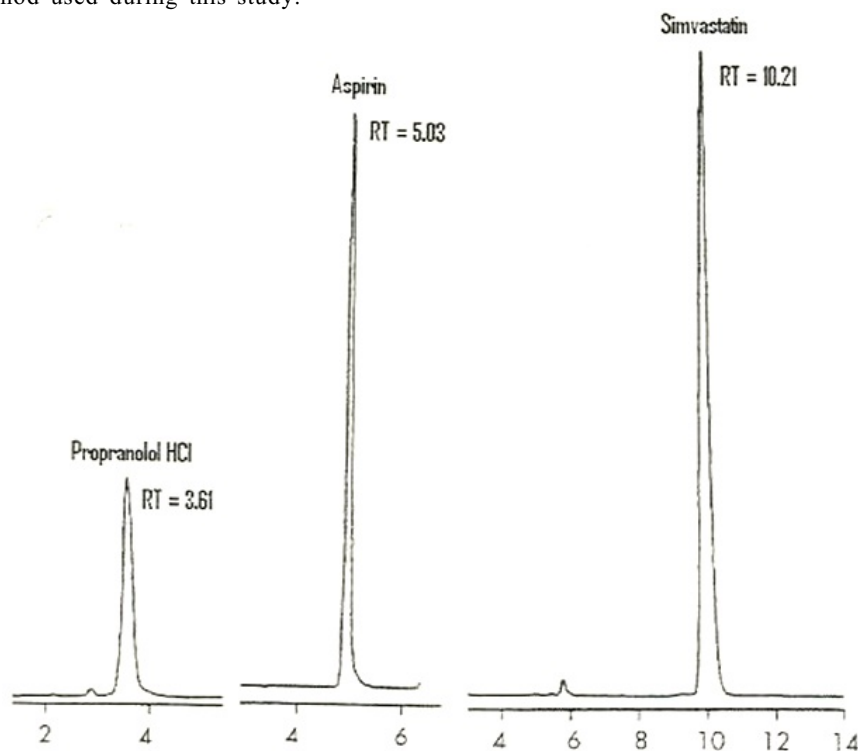


Fig. 5: Typical high performance liquid chromatograms of propranolol HCl, aspirin, and simvastatin, respectively from left to right.

Fig. 6 shows the dissolution profiles of aspirin, simvastatin, and propranolol HCl from their tablets in 0.1 N HCl at 37 °C. The amount dissolved % within two hours in 0.1N HCl of propranolol HCl reached 99 %, for aspirin reached 80 %, and for simvastatin was 24.5 %. These data of dissolution study indicates the possibility of formulation of the chosen three drugs in a single tablet with controlled release rate of each drug. The slow release of simvastatin because it is practically insoluble in water.

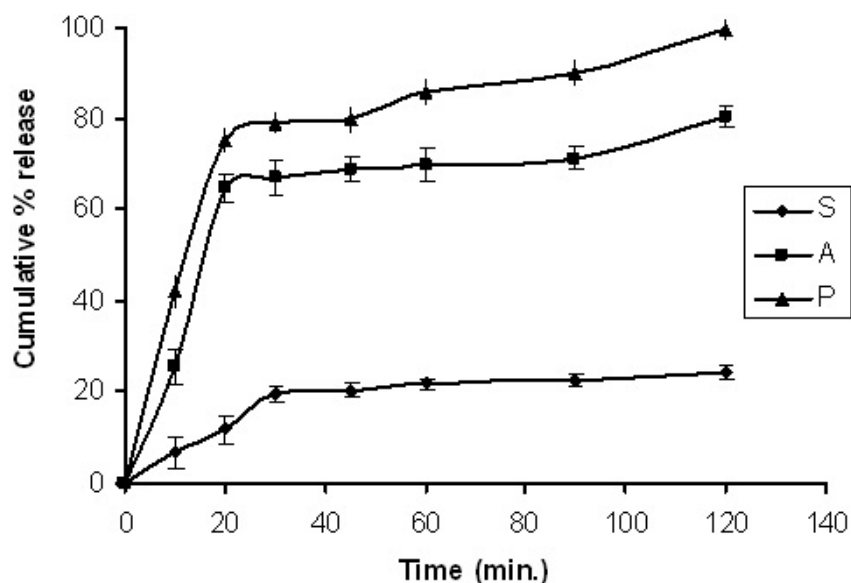


Fig. 6: Dissolution profiles of simvastatin (S), aspirin (A) and propranolol HCl (P) from triple tablets in 0.1 N HCl.

In conclusion, FT-IR spectra and DSC data of ternary mixture of aspirin, simvastatin and propranolol HCl revealed that there are no interactions between them in the solid state. It was found that HPLC is sensitive, reproducible and valid for determination of aspirin, simvastatin and propranolol HCl in presence of each other. Quality control tests of this single tablet dosage form containing the chosen three drugs revealed promising formula for improved patient compliance during treatment or prevention of cardiovascular diseases.

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