Simultaneous Spectrophotometric Determination of Clopidogrel Bisulfate, Atorvastatin Calcium Trihydrate and Aspirin by Double Devisor Ratio Derivative Spectrometric Method

Ahmed Jamal Ibrahim Al-Dulaimi, Khalaf F. Atiya Al-Samarraie, Eman Abdulwaheed Othman AL-Hitti

Abstract: This study uses two spectrophotometric simple, accurate and economic methods for the simultaneous determination of Clopidogrel Bisulfate(CLO), Atorvastatin Calcium Trihydrate (ATV) and Aspirin (ASP) in their pharmaceutical form. mhe first Method is th first ratio derivative and the second method is the second ratio derivative, double devisor.

The analytical aftermaths for each drug in the presence of the two others were as follows; CLO in presence of ATV and ASP showed Rec% (92.857-101.635)% and, (95.492-103.174)% , RSD % (0.000-0.363)% and (0.092-1.856)%,while for ATV in presence of CLO and ASP were Rec % (95.034-104.484%) and (95.090-103.174)%, RSD% (0.010-0.853)% and (0.647-1.749)%, and for ASP in presence of CLO and ATV Rec% (95.103-103.997)% and (96.107-103.091)%, RSD % (0.129-0.797)% and (0.094-0.878)%for the two methods respectively.

Applying the two methods for all the pre-mentioned drugs in their pharmaceutical forms was successful.

Keywords: Clopidogrel Bisulfate, Atorvastatin Calcium Trihydrate, Aspirin, First Ratio Derivative and Second Ratio Derivative.

INTRODUCTION

CLO or Methyl (－)-(R)-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate, hydrogen sulfate(1)is medically used to inhibit platelet aggregation(2)which decreases heart attacks, brain strokes(3), vascular disease(4). It is also used for arteriosclerosis patients to exclude Myocardial infarction in patients have history of such symptoms(5).Figure 1A shows molecular structure.
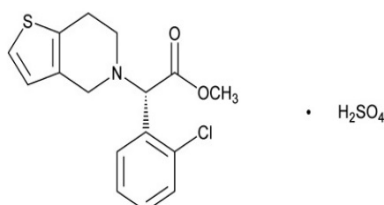

ATV or R-(R*,R*)]-2-(4-fluorophenyl)-b,d-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1Hpyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate(6) decreases LDL (7) which lessen

Mo Ahmed Jamal Ibrahim Al-Dulaimi, University of Samarra, College of Education, Department of Chemistry, Salah Al-Din, Iraq.
Khalaf F. Atiya Al-Samarraie, University of Samarra, College of Education, Department of Chemistry, Salah Al-Din, Iraq.
Eman Abdulwaheed Othman AL-Hitti, University of Samarra, College of Education, Department of Chemistry, Salah Al-Din, Iraq.

cholesterol in liver cells(8), reduces triglyceride levels in blood(9) and slightly raises up HDL level(11).fig. 1B shows molecular structure.
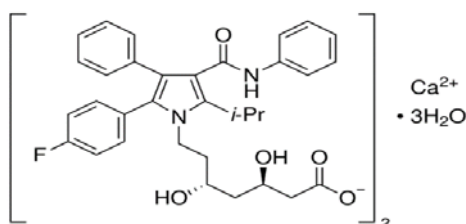
Finally, ASP or Acetylsalicylic acid (10) is used as pain relief(11), decreases temperature(12), decreases arthritis,(13), stops blood clotting(14), treat cold(15), menstrual cycle symptoms in women(16)and headache(17).Fig 1C shows molecular structure.
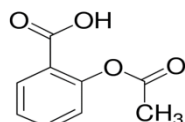
In 1990, the devisor ratio was applied for the first time to determine a constituent quantity of a solution without pre splitting its components (18). It also enables us in reducing spectrum overlapping (19). The method work is in dividing a mixture's spectrum of a solution on the spectrum of one of its constituents needs to be determined(20), therefore, the method was used to determine Tamsulosin Hydrochloride(21), Sulfamethoxazole, Famotidine, Domperidone(23). It also used to determine a drug compounded of Aspirin, Paracetamol and salicylic Acid (24). The HPLC –UV, UPLC-DAD, spectrum derivatives and RP-HPLC were used for determination of ASP, ATP and CLO (26.27).

The study aims to develop a method by which we simultaneously determine ASP, CLO and ATP via devisor ratio derivative and double devisor ratio.

The Practical Part

Chemical substances
A.   Pure substances.
   • CLO is made by Monovo, China.
   • ATV is made by Farmabios, Italy.
   • ASP is made by PHD Chamber, India.
B.   Pharmaceutical preperations
   • Tresprin A75 is made by Laborate, India
   • Dospin A75 is made by Ajanta Pharma, India.
   Each pharmaceutical preparation contains 75 mg of CLO and ASP and 10 mg of ATV.

Apparatuses

   • Shimadzu UV- visible -1800(Japan). The wavelength (190-400 nm) was used in the determination.
   • Centrifuge device.
   • Ultrasonic is made by Elma, Germany
   • Vortex shaker is made by Dragon lab., China.

Standard and Pharmaceutical Solutions Preparation

   • The standard solutions were prepared by disolving 0.1 g of ASP, ATV and CLO in (70:30 v/v) methanol and distilled water in a volumetric flask of 100 ml. Then, the same solvent was used to full the flask up to the mark. Later the working solutions were prepared as required.
   • Pharmaceutical Solution preparation
   A twenty capsules contents weigh 9.55 gm were taken, grounded and mixed using porcelain mortar, then a one capsule content 0.4775 gm has been taken. This quantity were put in 100 mL volumetric flask contains 65 mg of ATV where each component amount in the mixture has become 75 mg. The volume was completed to the mark with the same solvent, well shacked, sonicated for 10 minutes, centrifuged at 3000 cycle/minutes for 5 minutes and filtrated using syringe filter paper (0.45μm).
   • **Procedure** and **construction of calibration Curves**
   The solutions were prepared with concentrations of 10-27.5μg for ATV and ASP. CLO concentrations were 10-32.5 μg. The wavelengths were scanned between 190-400 nm, then the resulted absorption spectrum of the substances, quaternary and binary mixtures were stored on PC. The spectrum of quaternary mixture was devided on the spectrum of binary mixture of the two constant concentration

components (10μg), that was to get the ratio sectrum. These ratio spectrum were derived to get the first ration derivative which showed peak at 246 nm. The second ratio derivative showed two peaks at 258 and 280 nm for CLO.

ATV first ratio derivative showed three peaks at 248, 290 and 314 nm, while ATV second derivative showed 5 peaks at 242, 260, 284, 302 and 322 nm.

ASP first ratio derivative showed one peak at 240 nm, and its second tatio derivative hsowed two peaks at 228 and 280 nm. These peaks belong to modes of analysis; peak to base line, area under peak and peak to peak.

RESULTS AND DISCUSSION

Appropriate Solvent Choice

Many solvents were used to dissolve each drug components; distilled water, ethanol, methanol, acetonitrile or their mixtures. The results showed that methanol: distilled water (30:70 v/v) is the best solvent mixture.

Absorption Spectrums

Absorption spectra showed strong overlapping which makes their determination very difficult where CLO, ATV and ASP peak at 198 nm,240 nm and 200nm respectively. Figure 2 exhibits the absorption spectra for; (A)CLO, ATV and ASP mixture where their concentrations were 20 μg/mL, (B) CLO is 10 μg/mL, (C) ATV is 10 μg/mL and (D) ASP 25 μg/mL.

Figure 3 shows the ratio spectra for (A) CLO. (B) ATV. (C) ASP where their concentrations 32.5 μg/mL, 10 μg/mL and 27.7μg/mL respectively.Figure 4exhibits (A) first derivative ratio spectra,(B) second derivative ratio spectra for CLO 10-27.5 μg/mL, (C) first ratio derivative spectra, (D) second ratio derivative spectra for ATV 10-27.5μg/mL, (E) first ratio derivative spectra and (F) second ratio derivative spectra for ASP 10-32.5 μg/mL at wavelengths mentioned in table 1 for area under peak and peak to peak. All specra were scanned at Δλ=20 nm and scaling factor=1 nm.

Fig. 2   Fig. 3
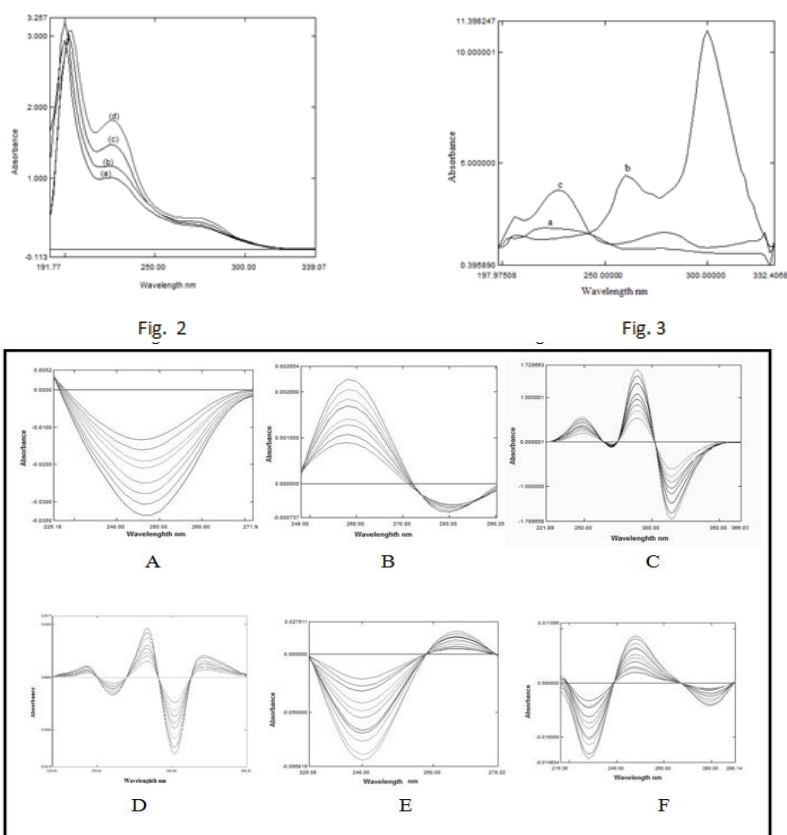

Figure 4: Shows (A) first ratio derivative spectra, (B) second ratio derivative spectra for CLO 10-27.5 μg/mL, (C) first ratio derivative spectra, (D) second ratio derivative spectra for ATV 10-27.5 μg/mL, (E) first ratio derivative spectra and (F) second ratio derivative spectra for ASP 10-27.5 μg/mL.

Table 1: Shows the analysis characteristics of ASP, ATV and CLO using modes of first and second ratio derivatives

Compound	Order of Derivative	Mode of Analysis	Linearty µg.mL-1	λ(nm)	R²	slope	LOD µg.mL-1	LOQ µg.mL-1
CLO	DD1	Peak Area	10-27.5	228-272	0.9974	-0.0303	0.1086	0.3583
		Peak to Base Line	10-27.5	246	0.9973	-0.0012	0.1090	0.3597
	DD2	Peak Area	10-27.5	248-270	0.9969	0.0012	0.0615	0.2030
		Peak to Base Line	10-27.5	258	0.9961	0.000078	0.0417	0.1375
		Peak to Base Line	10-27.5	280	0.9944	-0.0000009	0.3384	1.1167
		Peak to Peak	10-27.5	258+280	0.9972	0.00007	0.3143	1.0371
ATV	DD1	Peak Area	10-27.5	224-264	0.9932	0.4229	0.0307	0.1014
		Peak Area	10-27.5	276-302	0.9926	1.048	0.1311	0.4326
		Peak Area	10-27.5	304-366	0.9942	-1.4387	0.2289	0.7553
		Peak to Base Line	10-27.5	248	0.9982	0.0205	0.0290	0.0957
		Peak to Base Line	10-27.5	290	0.9933	0.0624	0.1148	0.3787
		Peak to Base Line	10-27.5	314	0.9928	-0.0638	0.1574	0.5195
	DD2	Peak Area	10-27.5	220-250	0.9932	0.0242	0.0272	0.0897
		Peak Area	10-27.5	252-268	0.9988	-0.0313	0.0237	0.0783
		Peak Area	10-27.5	270-290	0.9984	0.086	0.0944	0.3115
		Peak Area	10-27.5	292-312	0.9932	-0.1453	0.0974	0.3213
		Peak Area	10-27.5	314-354	0.9945	0.0679	0.1574	0.5194
		Peak to Base Line	10-27.5	242	0.9945	0.0016	0.0212	0.0701
		Peak to Base Line	10-27.5	260	0.9934	-0.0026	0.0248	0.0817
		Peak to Base Line	10-27.5	284	0.9982	0.0071	0.1007	0.3322
		Peak to Base Line	10-27.5	302	0.9933	-0.0111	0.0848	0.2797
		Peak to Base Line	10-27.5	322	0.9935	0.0031	0.1782	0.5882
		Peak to Peak	10-27.5	242+260	0.9986	-0.0011	0.0388	0.1282
		Peak to Peak	10-27.5	260+284	0.9976	0.0045	0.1556	0.5135
		Peak to Peak	10-27.5	284-302	0.9919	-0.004	0.0966	0.3188
ASP	DD1	Peak Area	10-32.5	226-256	0.9937	-0.0554	0.0388	0.1280
		Peak to Base Line	10-32.5	240	0.9968	-0.0028	0.0358	0.1183
		Peak Area	10-32.5	260-276	0.997	0.0075	0.7970	0.7891
	DD2	Peak Area	10-32.5	220-238	0.9972	-0.0053	0.0381	0.1258
		Peak to Base Line	10-32.5	228	1	-0.0004	0.0274	0.0906
		Peak Area	10-32.5	240-266	0.9975	0.0042	0.0180	0.0593
		Peak to Base Line	10-32.5	280	0.994	-0.0001	0.0784	0.2587

Limit of Detection and Limit of Quantification

The values of limit of detection for all modes of analysis were 0.0290-0.7970 μg/mL for the first ratio derivative 0.0180-0.3384 for second ratio derivative is while the limit of quantification values were 0.0957- 1.037μg/mL for first ratio derivative and 0.0593- 1.1167 μg/mL for second ratio derivative. These values were calculated as below:

$$\text{L. O. D} = \frac{3.3 * \text{S} * \text{Conc.}}{\text{X}}$$
$$\text{L. O. Q} = \frac{10 * \text{S} * \text{Conc.}}{\text{X}}$$

Whereas X= mean of measurments

S= standard deviation

C = the loe concentration of calibration

Accuracy and Precision

The Accuracy and precision of the methods were tested according to the international conference on harmonization (ICH).

Recovery percentage Rec% values for all calibration curves concentrations were 92.8571- 101.6355% and relative standard deviation RSD% values were 0.0000-0.3634% for first ratio derivatives, while for second ratio derivative Rec%values were 95.4921- 103.1746% and RSD% were 0.0929- 1.8561% for CLO drug.

The same test performed on ATV, it showed; Rec% 95.0348-104.4841% and RSD% 0.0106-0.8533% for first ratio derivative while the second ratio derivative Rec% 95.0908-103.1746% and RSD% 0.6476-1.7495%.

ASP test showed Rec%95.1038-103.9976% and RSD% 0.1293-0.7970% for the first ratio derivative. While for second ratio derivative Rec% was 96.1077-103.0918% and RSD% 0.0047-0.8783%.

Method Application

The single standard addition method was utilized in the determination of the two pharmaceutical preparations under study (Tresprin A75 and Dospin A75) by replicating each measurement seven times (n=7) for two concentrations of each pharmaceutical preparation, these two concentrations are within the calibration curve concentrations (25 and 27.5) μg/mL for CLO in the presence of 10 μg/mL ASP and ATV, (22.5 and 25) μg/mL of ATV in the presence 10 μg/mL of CLO and ASP and 15.27.5 μg/mL in the presence of 10 μg/mL of ATV and CLO. The results showed the two methods were applied successfully in the simultaneous determination of ASP, ATV and CLO. The Rec% and RSD% values for first and second ratio derivatives for the three components explained in table (2).

Table 1: Shows the Method Application of Tresprin A75 and Dospin A75

Compound	Order of Derivative	Rec%	RSD%
CLO		97.0863- 102.1583	0.0379-0.3759
ATV	DD1	97.6098- 104.8854	0.0369- 0.9349
ASP		97.0890 - 103.7153	0.0334 - 0.0924
CLO		95.1282 - 104.3590	0.0168- 0.7624
ATV	DD2	104.9104 - 96.3509	1.1228 - 0.0178
ASP		97.0749 - 102.7982	0.0092 - 0.3046

Conclusion

First ratio derivative and second ratio derivative are two spectrophotometric methods developed for the simultaneous estimation of CLO, ATV and ASP in their pharmaceutical preparations. Analysis modes; Peak to baseline, area under peak and peak to peak directly proportional with the concentration of each of the three drugs, therefore, these modes were utilized in the quantitative estimation at specific wavelengths.

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