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Simultaneous Estimation of Chlorpromazine hydrochloride and Carvedilol in Bulk and Pharmaceutical Dosage Forms Using HPLC

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Abstract

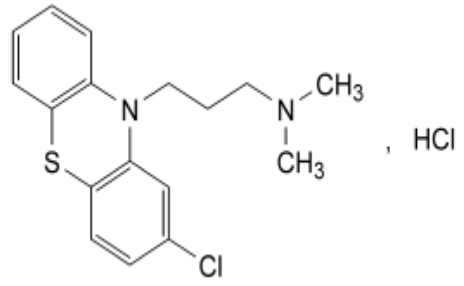
A simple, rapid and accurate HPLC method was developed and assessed for the simultaneous estimation of Chlorpromazine hydrochloride and Carvedilol in its bulk and Pharmaceutical tablets. The separation was obtained by using C18 ec (250 × 4.6 mm, 5µm) column with a mobile phase composing of acetonitrile: deionized water (50: 50 v/v, pH adjusted to 3.6 ± 0.03 with acetic acid) at flow rate of 1.5 mL.min^{-1}. The detection was carried out at 237nm, the retention time of CLO, and CARs were 1.56 and 3.84 min; respectively. The linearity range of 2-150 µg/mL and 1-150 µg/mL were selected for CLO and CAR; respectively. The correlation coefficients were found to be 0.9995 and 0.9999, and the limits of detection were found to be 0.6259µg/mL and 0.9365µg/mL for CLO and CAR; respectively. All the validation parameters were found to be within the acceptance limit. The developed HPLC method can be successfully used for the quantitative estimation of the two cited drugs in their formulation.

Keywords: Chlorpromazine Hydrochloride, Carvedilol, Pharmaceutical Tablet, HPLC

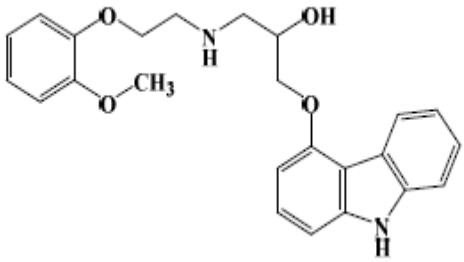
Introduction

Chlorpromazine hydrochloride (CLO), chemically called as 2-Chloro-10-[3-(dimethylamino) propyl]-phenothiazine monohydrochloride (Figure 1) (British Pharmacopoeia, 2013) is widely prescribed as an anti psychiatric drug (Diaz, 1997), used in the treatment of schizophrenia, and is also used to treat other diseases such as bipolar disorder, attention deficit hyperactivity disorder, anxiety before surgery, nausea and vomiting (Seeman, 1981), and it was first synthesized in the United Kingdom in 1950s (Gordon, 1964). CLO works on a variety of receptors in the central nervous system, producing anticholinergic, antidopaminergic, antihistaminic, and weak antiadrenergic effects (Seeman, 1981).

Carvedilol (CAR) chemically called as (2RS)-1-(9H-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl] amino] propan-2-ol. (Figure 2) (British Pharmacopoeia, 2013), is a nonselective beta-blocker/ alpha-blocker antihypertensive agent, widely used in the treatment of hypertension, congestive heart failure, cardiac arrhythmia, and angina pectoris (Tapas et al., 2012).



Chlorpromazine hydrochloride



Carvedilol

Fig. 1: The structures of chlorpromazine hydrochloride and carvedilol.

The literature has revealed several methods for the estimation of CLO and CAR in their pure and pharmaceutical formulations including voltammetry (Anfal, 2013; Yongnian et al., 2001; Bilal and Duygu, 2011) Potentiometry (Eman et al., 2012; Soleymanpour and Ghasemian, 2015) spectrophotometry (Sarah and Kasim, 2016; Israa, 2016; Laila, 2014; Sarmad et al., 2016; Shariti-Rad et al., 2014) and HPLC (Yaminia and Farajib, 2014; Zhang et al., 2007; Sánchez et al., 2005; Sarmad et al., 2016; Elezovic et al., 2015).

Experimental
Apparatus

Shimadzu UFLC system (Kyoto, Japan) equipped with LC-20AD solvent delivery pump; SIL20AC auto sampler unit; DGU-20A5 on-line vacuum degasser unit and a SPD20A Shimadzu UV-Vis detector were used in this study. Chromatographic analysis was performed on a NUCLEODUR® 100-5 C18 ec (250 × 4.6 mm,

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5µm particle) (MACHEREYNAGEL-Germany) with isocratic conditions. The mobile phase consisted of acetonitrile: deionized water (50:50v/v, pH adjusted to 3.6±0.03 with acetic acid) at a flow rate of 1.5 mL.min^{-1}, and the quantification was achieved at 237 nm.

Reagents and Materials

Chlorpromazine hydrochloride and carvedilol standard powders with pure form (99.99%) were provided as a gift from the State Company for Drug Industries and Medical Appliances Samarra, Iraq (S.D.I).

The pharmaceutical formulations used in this study were Largactil® 100mg/tablet (Aventis, France) and Neurazine® 100 mg/tablet (Misr Co., Egypt), Carvidol® 25 mg/tablet (Pharma International, India) and Carvedilol® 6.25 mg/tablet (EMESSA, Syria), and were procured from local pharmacies.

HPLC grade acetonitrile (99.9 %), HPLC grade water, and (99-100%) acetic acid were from Sigma Aldrich.

Standard Drugs Solutions

1000 µg. mL^{-1} standard solutions of CLO and CAR were prepared separately in different 20mL standard flasks. These solutions were prepared by dissolving exactly 20mg of each drug in acetonitrile, and the volume was made up to the mark with some solvents. The stock solutions were stored at 5°C, and were protected from the light. Working solutions were freshly prepared by subsequent dilutions with acetonitrile.

Solutions of Pharmaceutical Preparations

Ten formulated tablets from each drug were separately weighed, and finely powdered. Quantities of powder were equivalent to 0.01910g, 0.04602 g 0.1042 and 0.1519 (each containing 0.0100 g of the drug) for Largactil® 100mg, Neurazine®100mg, Carvidol® 25mg, and Carvedilol 6.25mg respectively, and were transferred to 10mL volumetric flask and dissolved in acetonitrile, and completed to the mark with the same solvent. The sample solution, then was shaken well and filtered through Whatman filter paper. More dilute solutions were prepared by the suitable dilution with acetonitrile.

Preparation of Calibration Standard drugs

The mixture, containing different concentrations of the CLO and CAR, was prepared by serial dilutions at the concentration levels of (1- 150) µg. mL^{-1} for the studied drugs.

Results and Discussion

HPLC method
Wavelength selection

The wavelength selection to investigate the appropriate wavelength for the detection of CLO and CAR chromatographic bands, and (10µg.mL^{-1}) solution for the cited drugs were prepared in acetonitrile. Each solution was scanned by UV-visible spectrophotometer for the range of 200 - 400 nm. The overlapped UV spectra showed that the two drugs showed approximately equal reasonable values of absorbance at 237nm. Therefore, the wavelength 237nm was selected for quantification and monitoring the drugs in HPLC system (see Figure.2).

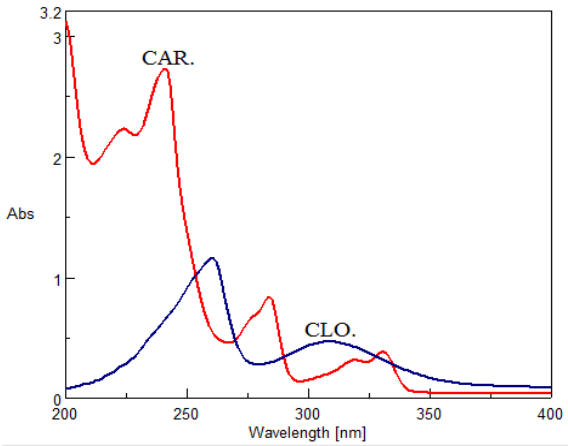

Fig. 2: UV spectra of CLO and CAR drugs (10 µg. mL^{-1}) in acetonitrile against acetonitrile.

Mobile phase selection

After several trials by testing different mobile phases of acetonitrile: the deionized water (50: 50 v/v, pH adjusted to 3.6 ±0.05 with acetic acid) was chosen as the mobile phase, which gave good resolution and peak.

Selection of mobile phase ratio

To obtain good separation, different ratios of acetonitrile: deionized water v/v, pH adjusted to 3.6 ±0.05 with acetic acid) (8:2, 6:4, 7:3, 2:8, 4:6, 3:7, 5:5) were tried, 5:5% v/v was chosen as the optimal ratio of the mobile phase since it gave well defied chromatographic bands with reasonable analysis time, and it was used for the subsequent work.

Flow rate of mobile phase selection

To obtain a short analysis time and preventing solute band diffusion, the flow rate of the mobile phase was changed from 0.8 to 1.7 mL.min^{-1}, the optimal flow rate was selected with the most efficient separation using a 1.5 mL. minute^{-1}.

Temperature selection

The effect of column temperatures in the range of (25 to 50) ºC on the retention time of drugs was investigated, the optimum column temperature was found to be 35ºC. At this temperature, good shape and good resolution values of the separated bands of

the drugs were obtained. At the higher temperature, the resolution as well as the column efficiency was reduced due to the reduction in the interaction of the drugs with the stationary phase.

Flow rate selection

The aim of choosing the optimum flow rate was to obtain short analysis time and preventing solute band diffusion, which in turn led to the high column efficiency (R. O. Hassan, 2001). The flow rate of the mobile phase had an important effect on the analyte retention time. The higher flow rates led to a shorter retention time because the eluent carried the drugs through the column faster after the desorption and vice versa. Clear from the results, when the flow rate of the mobile phase was changed from 0.8 to 1.5 mL.min^{-1} causing the time analysis to decrease to 16 6min.

Effect of injection volume

The optimization of injection volume on CLO and CAR analysis was studied over the range of (5 - 30) µL, the results showed the volume injection appeared significantly different in the peak area and peak height, this was related to the amount of analyte passed through the column. The injection volume should be adequate in a way that the peak area of the smallest concentration could be easily measured (S. Mogatle, 2008), therefore; 20 µL was chosen to be the best volume injection since it gave acceptable resolution values and reasonable analysis time.

The developed method was found to be rapid as CLO and CAR eluted out at 1.56, and 3.84 minutes; respectively. The descriptive chromatogram of mixed standard solution of (10 µg. mL^{-1}) for both drugs CLO and CAR is shown in Figure 3.

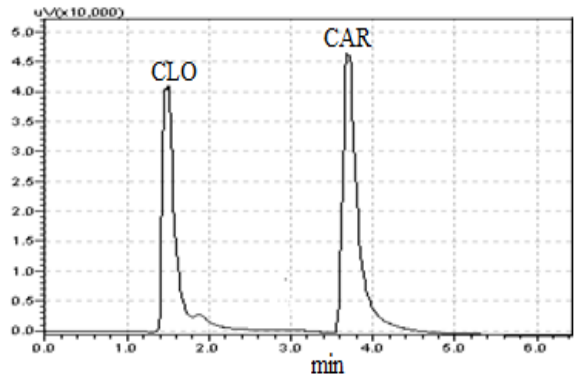

Fig. 3: Chromatogram for linearity standard solution of two drugs under optimized HPLC conditions.

Calibration graphs

According to the experimental conditions, the linearity was examined in the concentration range of (1 -150) µg. mL^{-1} for the mixture of CLO and CAR. The obtained Calibration graphs done by plotting concentration (µg. mL^{-1}) versus peak area and peak height for the studied drugs are shown in Figure 4. And, Table (1) shows the linearity parameters of (CLO) and (CAR) by the suggested method.

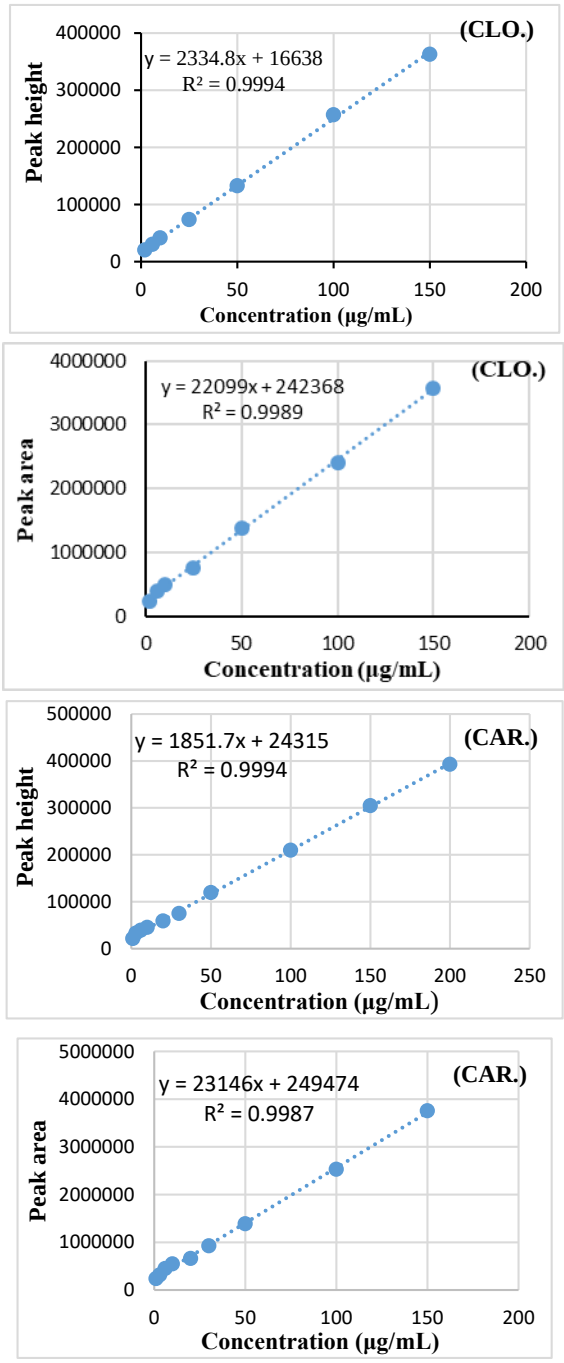

Fig. 4: The calibration graphs for sited drugs, concentration against peak height or peak area.

Table 1- Linearity parameters of (CLO) and (CAR) by the suggested method.

Drug	Concentration (µg/mL)	Calibration graph	r	slop	LOD (µg/mL)	LOQ (µg/mL)
CLO	2- 150	Peak height	0.9994	2334.80	0.2066	0.6259
	2- 150	Peak area	0.9989	22099.00	0.1837	0.5566
CAR	1- 150	Peak height	0.9994	1851.70	0.3091	0.9365
	1- 150	Peak area	0.9987	23146.00	0.2693	0.8149

Accuracy and precision

Under recommended procedure, the accuracy (%RSD) and the precision (%RE) of the obtained results for the studied drugs were evaluated. Each drug was determined at two different concentrations in three replicates during the same day. The results in table (2) indicate good accuracy and precision of the proposed method at the studied concentration levels.

Table 2- Precision and accuracy of the suggested method.

Drug	Injected (µg/mL)	Conc. calculated from peak height				Conc. Calculated from peak area			
		Mean*	SD	RSD %	RE%	Mean*	SD	RSD %	RE%
CLO	10	9.8896	0.1460	1.4762	-1.1040	9.9539	0.0856	0.8599	-0.4610
	50	50.0280	0.1099	0.2196	0.0560	50.0475	0.0777	0.1552	0.0950
CAR	10	9.9258	0.0302	0.3042	-0.7420	9.9541	0.0324	0.3254	-0.4590
	50	50.0267	0.0817	0.1633	0.0534	50.0243	0.1033	0.2064	0.0486

*Average of three measurements.

Application

The suggested method was successfully applied for the estimation of chlorpromazine hydrochloride and carvedilol drugs in the commercially available tablets, the quantitative determination of the sited drugs was carried out following the suggested HPLC procedure.

From the pharmaceutical tablets, the stock solution of each drug at two concentrations was prepared by serial dilution (10 and 50) µg/mL, and then injected three times into the HPLC system under the optimum conditions. The amount of drugs was calculated accurately, and the results obtained were recorded in Table (3). The good agreement between these results and known values indicated the successful applicability of the proposed method for the determination of chlorpromazine hydrochloride and carvedilol in their pharmaceutical preparations. And, Table (4) indicates the application of the method to the drugs' concentration measurements in different pharmaceutical preparations.

Table 3- Application of the method to the drugs, and concentration measurements in different pharmaceutical preparations.

Sample of pharmaceutical tablet	Conc. (µg/mL)		Conc. calculated from peak height		
	Taken	Found*	Weight found* (mg/tablet)	Recovery %	RSD %
CLO. (France) 100mg/tablet	10	10.3107	103.1070	103.1070	0.1840
	50	50.8237	101.6474	101.6474	0.3471
CLO. (Egypt) 100mg/tablet	10	10.5537	105.5370	105.5370	1.3660
	50	50.8701	101.7402	101.0740	0.6359
CAR. (India) 25 mg / tablet	10	9.9644	24.9110	99.6440	0.7824
	50	49.9335	24.9667	99.8670	0.0629
CAR. (Syria) 6.25 mg / tablet	10	10.1796	6.3622	101.7960	1.5815
	50	50.5976	6.3247	101.1952	1.1099

*Average of three measurements.

Table 4- Application of the method to the drugs' concentration measurements in different pharmaceutical preparations.

Sample of pharmaceutical tablet	Conc. (µg/mL)		Conc. calculated from peak height		
	Taken	Found*	Weight found* (mg/tablet)	Recovery %	RSD %
CLO. (France) 100mg/tablet	10	10.6017	106.0170	106.0170	0.8451
	50	50.2940	100.5880	100.5880	0.8936
CLO. (Egypt) 100mg/tablet	10	9.9572	99.5720	99.5720	0.6427
	50	49.8774	99.7548	99.7548	0.3179
CAR. (India) 25 mg / tablet	10	9.9736	24.9340	99.7360	0.9264
	50	50.1834	25.0917	100.3668	0.5049
CAR. (Syria) 6.25 mg / tablet	10	10.4051	6.5031	104.0510	0.6103
	50	50.2059	6.2757	100.4118	0.7410

*Average of three measurements.

Conclusion

A simple, specific, accurate and reproducible high performance liquid chromatography method was developed for the simultaneous estimation of Chlorpromazine hydrochloride and Carvedilol propionate in their pure forms. Hence, the developed HPLC method can be useful for the quantitative determination of Chlorpromazine hydrochloride and Carvedilol in their pharmaceutical formulations.

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