Chemistry and Materials Research
ISSN 2224- 3224 (Print) ISSN 2225- 0956 (Online)
Vol.8 No.3, 2016

Simple RP-HPLC Method for Estimation of Furosemide, Carbamazepine, Diazepam and Carvedilol in Bulk and Pharmaceutical Dosage Forms

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Abstract
A simple reverse-phase high performance liquid chromatographic method for the simultaneous analysis (separation and quantification) of furosemide (FURO), carbamazepine (CARB), diazepam (DIAZ) and carvedilol (CARV) has been developed and validated. The method was carried out on a NUCLEODUR® 100-5 C18ec column (250 x 4.6 mm, i. d.5µm), with a mobile phase comprising of acetonitrile: deionized water (50: 50 v/v, pH adjusted to 3.6 ±0.05 with acetic acid) at a flow rate 1.5 mL.min^{-1} and the quantification was achieved at 226 nm. The retention times of FURO, CARB, DIAZ and CARV were found to be 1.90 min, 2.79 min, 5.39 min and 9.56 min respectively. The method was validated in terms of linearity, accuracy, precision, limit of detection and limit of quantitation. The developed method was successfully applied for the estimation of furosemide, carbamazepine, diazepam and carvedilol in bulk and in pharmaceutical dosage forms.
Keywords: RP-HPLC, Furosemide, Carbamazepine, Diazepam and Carvedilol

1. Introduction

Furosemide (FURO) is a loop diuretic mainly used for the treatment of hypertension and edema (Sarrafi *et al*. 2010) chemically is 4-Chloro-2-[(furan-2-ylmethyl) amino]- 5-sulfamoylbenzoic acid.

Carbamazepine (CARB) is a lipophilic tricyclic compound used in treatment of epilepsy and neuropathic pain (Moshé 2009) chemically it is 5H-dibenzo [b,f]azepine-5-carboxamide.

Diazepam (DIAZ) chemically is 7-chloro-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one and is used as anxiolytic, hypnotic and muscle relaxant (Calcaterra and Barrow 2014).

Carvedilol (CARV) 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) chemically it is (2RS)-1-(9H-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl] amino] propan-2-ol. Structural formulas of FURO, CARB, DIAZ and CARV are given in Figure 1.

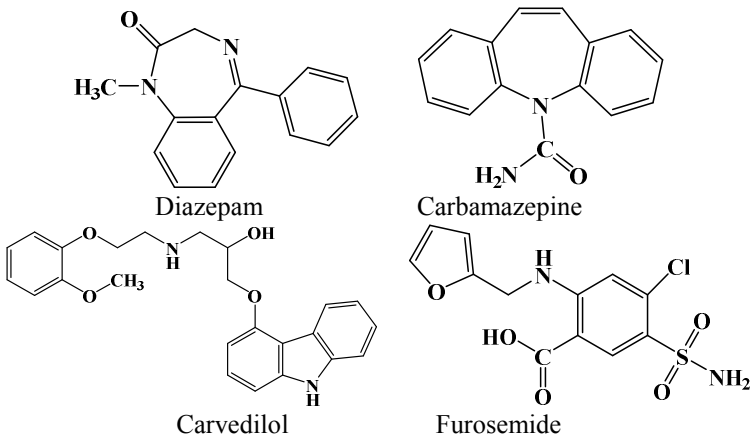

Figure 1. The chemical structures of furosemide (FURO), carbamazepine (CARB), diazepam (DIAZ) and carvedilol (CARV).

Review of literature revealed that a large number of analytical methods have been used to quantify CARV, FURO, CARB and DIAZ in their bulk and pharmaceutical formulations as well as in biological fluids including potentiometry (Ghorbani *et al*. 2014, Lee *et al*. 2014 and Soleymanpour & Ghasemian 2015), voltammetry (Pan *et al*. 2014, Hasanzadeh *et al*. 2014), GC-MS (Yilmaz & Arslan 2011, Zaporozhets *et al*. 2012 and Zhang *et al*. 2014), flow injection technique (Biparva *et al*. 2014 and Han *et al*. 2013), spectrophotometry (Harshad & Sugandha 2015, Rad *et al*. 2014, Mohamed *et al*. 2014 and Tharpa *et al*. 2010) and high performance liquid chromatography (Thejaswini & Gurupadayya 2014, Elezovic *et al*. 2015, Soltani & Jouyban 2011 and Baranowska & Kowalski 2011).

2. Experimental

2.1 Reagents and materials

Furosemide, carbamazepine, diazepam, and carvedilol reference substances were obtained as gift samples from the State Company for Drug Industries and Medical Appliances Samara-Iraq (SDI). HPLC grade acetonitrile (99.9 %) was from Sigma Aldrich (Baghdad), HPLC grade water was obtained from ROMIL (Baghdad), and (99-100%) acetic acid was purchased from Sigma Aldrich (Baghdad).

Pharmaceutical formulations Lasix 40 mg/tablet (SWI, France and SDI, Iraq), Tegretol® 2% (NOVARTIS, Switzerland), Carbamazepine 200 mg/tablet (TAVER, Cyprus), VALIAPAM 2 mg / tablet (SDI, Iraq), Valiapam 10 mg / 2mL (ALSAVAL, Syria), Carvidol® 25 mg / tablet (Pharma International, India) and Carvedilol 6.25 mg / tablet (EMESSA, Syria) were obtained from local pharmacies.

2.2 Instrumentation

Shimadzu UFLC system (Kyoto, Japan) equipped with LC-20AD solvent delivery pump, SIL-20AC auto sampler unit, DGU-20A_5 on-line vacuum degasser unit and a SPD-20A Shimadzu UV-Vis detector. Chromatographic analysis was performed on a NUCLEODUR® 100-5 C18 ec (250 × 4.6 mm i.d. 5µm particle) (MACHEREY-NAGEL Germany) with isocratic conditions. The mobile phase consist of acetonitrile: deionized water (50: 50 v/v, pH adjusted to 3.6 ± 0.05 with acetic acid) at a flow rate of 1.5 $mL.min^{-1}$ and the quantification was achieved at 226 nm.

2.3 Preparation of stock and working standard solutions

1000 $\mu g.mL^{-1}$ stock standard solutions of furosemide, carbamazepine and carvedilol were prepared separately in different 50 mL volumetric flasks by dissolving exactly 50 mg of each drug in acetonitrile, and diluted to the mark with acetonitrile. The stock solutions were protected from light and were stored at 4 ºC. Further dilution was done to get working standard solution (100 $\mu g.mL^{-1}$) of each drug.

2.4 Preparation of drugs in pharmaceutical formulations

2.4.1 Tablets

Ten tablets from each drug were separately weighed and powdered. A portion of the powder equivalent to 0.0380 g and 0.0400 g for Lasix (FURO) (Iraq, France) respectively, 0.3310 g and 0.1520 g for Carvedilol (CARV) (Syria, India) respectively, 0.0130 g for Carbamazepine (Cyprus) and 0.6010 g for Valiapam (DIAZ) (Iraq) were weighed, dissolved in about 8 mL of acetonitrile in a separate 10 mL volumetric flask. The solutions were sonicated for a minimum 10 min with intermittent shaking and diluted to mark with acetonitrile to get 1000 $\mu g.mL^{-1}$. Then after, each solution was filtered through Whatman filter paper No. 41 and stored as the standard stock solutions for further dilution in subsequent use.

2.4.2 Ampoule

The 1000 $\mu g.mL^{-1}$ Valiapam (DIAZ) solution was prepared by quantitatively transferring the content of 1 ampoule (10 mg /2 mL) to 10 mL volumetric flask, diluted to the mark with acetonitrile and stirred for 5 min for complete dissolution (mixing, homogenous) the drug. The resulted solution was filtered and further diluted with acetonitrile before its application to HPLC system for analysis.

2.4.3 Oral suspension

The content of one container of Tegretol (CARB) oral suspension (100 mg /5 mL) (2 %) was mixed well and 0.5 mL of the suspension was quantitatively transferred into 10 mL volumetric flask and dissolved in 10 mL of acetonitrile, shaken well and sonicated for 5 min then made up to the mark with acetonitrile to get 1000 $\mu g.mL^{-1}$. An aliquot of the resulted filtered solution was then treated as done in recommended procedure.

2.5 Preparation of samples for linearity

In order to ascertain that there is a direct proportional relationship between each analyte response and its concentration, mixture solutions containing different concentrations of FURO, CARB, CARV and DIAZ, were prepared by serial dilutions in a concentration rang of (1-100) $\mu g.mL^{-1}$ for each of the studied drugs.

3. Results and discussion

3.1 RP-HPLC method

The proper wavelength that gave approximately equal reasonable values of absorbance was selected by scanning the standard solutions of the four analytes (10 $\mu g.mL^{-1}$ of each drug) in the wavelength range of 200 - 400 nm. Figure 2 shows the overlaid UV spectra of the four medications from which the wavelength 226 nm was chosen for the HPLC detection and quantitation of the cited drugs.

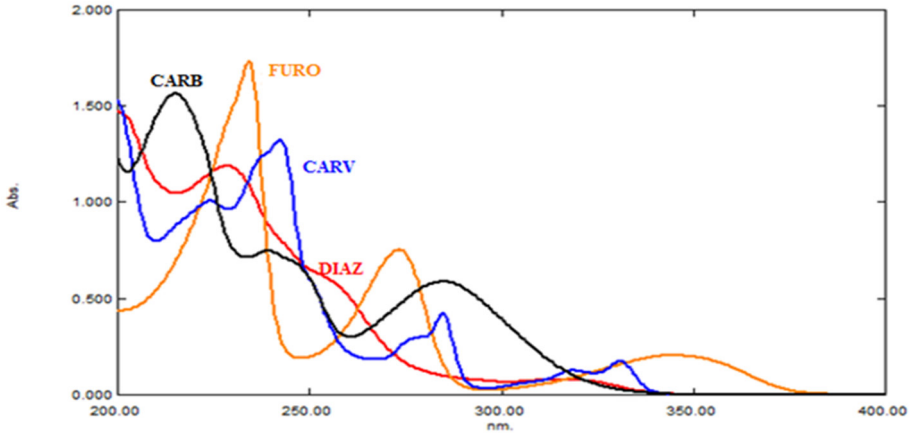

Figure 2. Overlaid UV spectra of the studied drugs (10 µg.mL^{-1}) in acetonitrile against acetonitrile as a blank.

Different mobile phases were tested to accomplish the simultaneous separation of the four analytes. A mobile phase consisting of acetonitrile: deionized water (50: 50 v/v, pH adjusted to 3.6 ±0.05 with acetic acid) was chosen for this purpose, after comparing the baselines, resolution and peak shapes under the various conditions. The most efficient separation was acheived by using a flow rate of 1.5 mL.min^{-1}, column temperature of 40 ^{o}C, and injection volume of 10 µL, since it gave acceptable resolution values and reasonable analysis time. The developed method was found to be rapid as FURO, CARB, DIAZ, CARV eluted out at 1.90, 2.79, 5.39, and 9.56 minutes respectively and the. The descriptive chromatogram of mixed standard solution of FURO, CARB, DIAZ (20 µg.mL^{-1} for each) and CARV (40 µg.mL^{-1}) is shown in Figure 3.

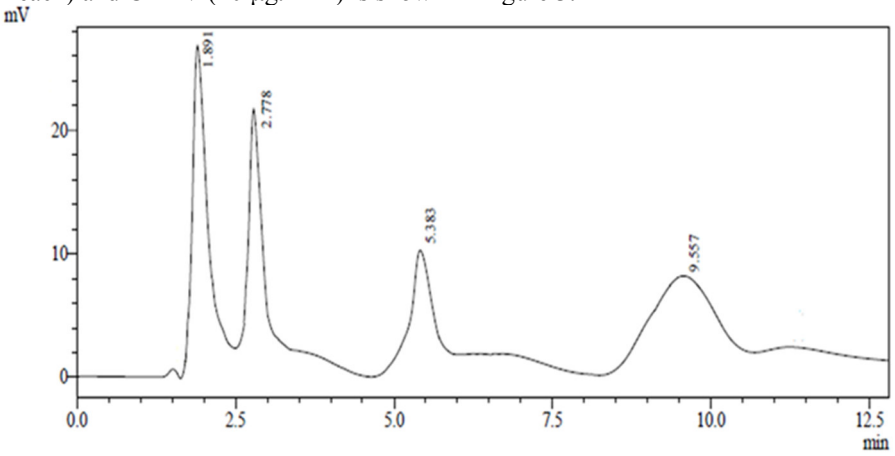

Figure 3. Chromatogram of standard solution of FURO, CARB, DIAZ and CARV.

3.2 Linearity and range

To determine the linearity of the proposed method, a series of mixed standard solutions of FURO, CARB, DIAZ and CARV were prepared and 10 µL of each solution was injected into the HPLC system. Calibration curves were obtained by plotting concentration (µg.mL^{-1}) vs. peak area and peak height for FURO, CARB, DIAZ and CARV (Figure 4).

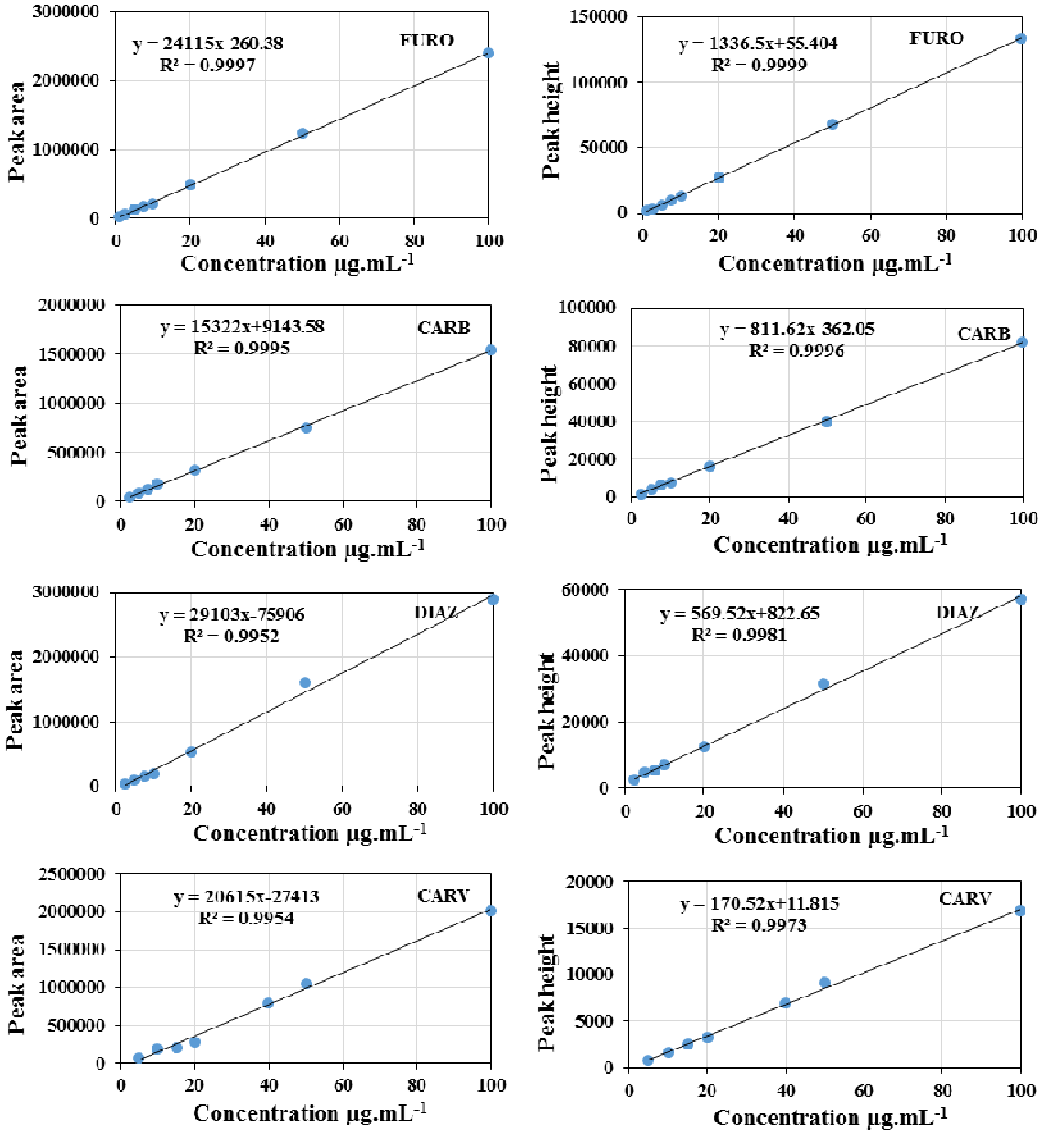

Figure 4. Calibration curves, concentration (µg.mL^{-1}) vs. peak area and peak height for FURO, CARB, DIAZ and CARV.

The linearity range, regression equation, correlation coefficient, limit of detection and limit of quantitation are given in Table 1.

Table 1. Linearity parameters for FURO, CARB, DIAZ and CARV assay method.

Drug	Linearity range (µg.mL^{-1})	Calibration curve	r	Slope	Intercept	LOD* (µg.mL^{-1})	LOQ+ (µg.mL^{-1})
FURO	1-100	Peak area	0.9999	24115	260	0.115	0.348
		Peak height	1.0000	1337	55	0.074	0.225
CARB	2.5-100	Peak area	0.9998	15322	9144	0.210	0.636
		Peak height	0.9998	812	362	0.171	0.519
DIAZ	2.5-100	Peak area	0.9976	29103	75906	0.475	1.440
		Peak height	0.9991	570	823	0.462	1.399
CARV	5-100	Peak area	0.9977	20615	27413	0.950	2.877
		Peak height	0.9988	171	12	0.528	1.599

3.3 Accuracy and precision

Accuracy of the RP-HPLC method was tested by determining the values of relative error percentage (RE %) and the precision was evaluated by calculating the values of relative standard deviation percentage (RSD %) of the obtained results for the studied drugs. Three replicate analyses were carried out for each drug at two different

levels of concentrations within the linearity range for each drug. The obtained results indicate good accuracy and precision of the recommended procedure at the studied concentration levels (Table 2).

Table 2. Accuracy and precision of the RP-HPLC method.

Drug	Injected ($\mu g.mL^{-1}$)	Calculated Conc. using peak area			Calculated Conc. from peak height		
		Mean*	RSD%	RE%	Mean*	RSD%	RE%
FURO	20	19.9346	1.167	-0.327	20.221	0.7229	1.107
	80	81.056	0.9628	1.32	80.96	0.9732	1.2
CARB	20	19.8258	1.5263	-0.871	19.054	1.5356	-4.729
	80	78.4613	1.5018	-1.923	82.664	1.1275	3.33
DIAZ	20	19.0398	2.0011	-4.801	20.339	2.7821	1.6951
	80	79.2053	2.6548	-0.993	80.754	1.3357	0.9429
CARV	20	19.3214	3.9056	-3.393	20.981	2.2751	4.9066
	80	81.3142	3.0715	1.6428	81.93	1.9848	2.4127

*Average of three measurements.

4. Application

The quantitative determination of FURO, CARB, DIAZ and CARV in commercially available pharmaceutical preparations (tablets, syrup and ampoule) was carried out following the proposed HPLC procedure. The Representative chromatogram of mixed pharmaceutical solution of FURO, CARB, DIAZ (20 $\mu g.mL^{-1}$ for each) and CARV (40 $\mu g.mL^{-1}$) is shown in Figure 5.

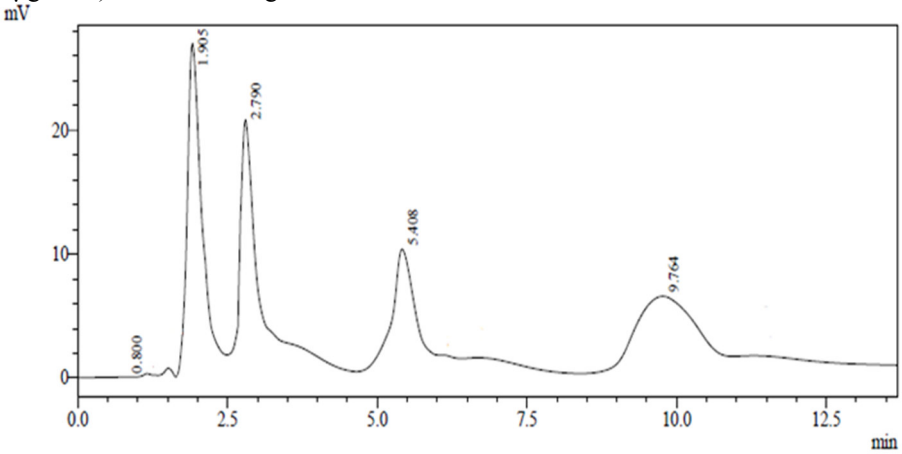

Figure 5. Representative chromatogram of FURO, CARB, DIAZ and CARV in pharmaceutical formulations.

Two concentration levels for each drug were separately injected in triplicate into the HPLC system and analyzed under the optimum conditions. The obtained values of recovery percentage (Tables 3 and 4) revealed the reliability of the recommended method for the determination of the cited drugs in their pharmaceutical formulations.

Table 3. Estimation of FURO, CARB, DIAZ and CARV in their pharmaceutical preparations via peak area.

Sample	Conc. (μg.mL^{-1})		Calculated Conc. using peak area		
	Taken	Found	Weight* found (mg/dosage)	Recovery %	RSD %
FURO (Iraq) tablet 40 mg	20	21.313	42.622	106.6	1.628
	80	83.845	41.923	104.8	1.247
FURO (France) tablet 40 mg	20	20.453	40.906	102.3	1.201
	80	82.699	41.349	103.4	1.080
CARB (Cyprus) tablet 200 mg	20	19.493	194.930	97.5	1.131
	80	78.570	196.425	98.2	1.294
CARB (Switzerland) syrup 100 mg	20	19.355	96.775	96.8	1.075
	80	75.325	94.156	94.2	0.987
DIAZ (Iraq) tablet 2 mg	20	20.339	2.034	101.7	1.548
	80	78.693	1.967	98.4	1.066
DIAZ (Syria) ampoule10mg	20	20.304	10.152	101.5	1.267
	80	82.323	10.290	102.9	0.942
CARV (Syria) tablet 6.25 mg	20	21.277	6.649	106.4	2.348
	80	83.279	6.506	104.1	2.075
CARV (India) tablet 25 mg	20	18.467	23.084	92.3	2.540
	80	74.885	23.402	93.6	1.774

*Average of three measurements.

Table 4. Estimation of FURO, CARB, DIAZ and CARV in their pharmaceutical preparations via peak height.

Sample	Conc. (μg.mL^{-1})		Calculated Conc. using peak height		
	Taken	Found	Weight* found (mg/dosage)	Recovery %	RSD %
FURO (Iraq) tablet 40 mg	20	19.850	39.700	99.3	1.447
	80	82.408	41.204	103.0	1.070
FURO (France) tablet 40 mg	20	20.289	40.578	101.4	0.698
	80	78.004	39.002	97.5	0.681
CARB (Cyprus) tablet 200 mg	20	18.807	188.070	94.0	1.043
	80	74.803	187.008	93.5	1.237
CARB (Switzerland) syrup 100 mg	20	18.240	91.201	91.2	1.008
	80	71.024	88.780	88.8	0.946
DIAZ (Iraq) tablet 2 mg	20	21.503	2.150	107.5	1.282
	80	77.565	1.940	96.9	1.040
DIAZ (Syria) ampoule10mg	20	20.995	10.498	105.0	1.147
	80	77.480	9.685	96.9	0.931
CARV (Syria) tablet 6.25 mg	20	21.315	6.661	106.6	2.696
	80	85.010	6.641	106.3	1.680
CARV (India) tablet 25 mg	20	20.449	25.561	102.2	2.304
	80	75.070	23.460	93.8	1.307

*Average of three measurements.

5. Conclusion

This study describes a simple, rapid, precise and accurate RP-HPLC method for the simultaneous identification and quantification of FURO, CARB, DIAZ and CARV in bulk and pharmaceutical preparations as well. The suggested method gave acceptable resolution between the four compounds in a short period below 10 minutes and can be applied for analysis of regular quality control samples of the investigated drugs.

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