Quantitative Determination of Four Angiotensin-II-Receptor Antagonists in Presence of Hydrochlorothiazide by a Gradient Technique HPLC in their Pharmaceutical Preparations

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Abstract

Losartan potassium, Valsartan, Telmisartan and Irbesartan are angiotensin-II-receptor antagonists (ARA II) group which used in treatment of hypertension alone or in combination with other drugs mainly Hydrochlorothiazide. A gradient HPLC method was developed for the assay of several ARAII in presence of Hydrochlorothiazide. The method was performed by reversed phase high performance liquid chromatography using a mobile phase 0.025 M potassium dihydrogen phosphate (pH 6.0): acetonitrile =80:20% with detection at 220 nm on an ACE C18 column (250 mm × 4.6 mm, 5 μm) at flow rate 1.5 ml/min in a gradient manner. The proposed method was validated in terms of linearity, accuracy, precision and limits of detection and quantitation.

Keywords: Hydrochlorothiazide; Losartan potassium; Irbesartan; Valsartan; Telmisartan

Introduction

Angiotensin antagonists are the first major innovation in essential hypertension management as a first-line treatment. Angiotensin II receptor antagonists (ARA II) have been developed to specifically and selectively block the AT1 receptor of the rennin angiotensin system by displacing angiotensin II from it [1]. Losartan potassium, Telmisartan, Irbesartan and Valsartan are highly selective, non-peptide angiotensin-II receptor antagonists (ARA-II). They are effective agents for the treatment of hypertension and heart failure either alone or together with diuretics or recently with other antihypertensive drugs [2]. So, it is necessary to develop a validated analytical method for assay of ARAII in combination with hydrochlorothiazide in its pharmaceutical preparations. Literature review revealed that USP described RP-HPLC methods for assay of Losartan potassium, Valsartan and ion pair HPLC for Irbesartan and Telmisartan. It described gradient RP-HPLC methods for assay of Losartan potassium, Valsartan in combination with hydrochlorothiazide and a gradient ion pair HPLC for Irbesartan in combination with hydrochlorothiazide [3]. BP described a potentiometric titration for assay of Losartan potassium, Irbesartan, Telmisartan and Valsartan [4]. Some methods have been published for simultaneous determination of studied ARA-II-drugs separately in combination with hydrochlorothiazide in its pharmaceutical preparations [5-15]. EIPICO Company described a RP-HPLC method for analysis of Losartan potassium and hydrochlorothiazide (50/12.5 mg) tablets. Analysis was performed on a Hypersil C18 column using mobile phase consisted of a mixture of acetonitrile and phosphate buffer (pH 3.5; 0.05 M) (50:50% v/v) and detector was set at 220 nm [16] other methods have been reported for determination of only ARAII drugs [17-19]. Only one method has been established to assay ARAII and hydrochlorothiazide by electrophoresis [20] but this technique is less available in pharmaceutical companies than HPLC-UV and more expensive so, it is preferable that the developed method is HPLC-UV, the most spreadable apparatus in pharmaceutical companies. Our recent method is characterized by a simplicity, accuracy, preciseness and sensitivity.

Hydrochlorothiazide is 2H -1, 2, 4-Benzothiadiazine-7-sulfonamide, 6-chloro-3, 4-dihydro 1, 1-dioxide; Hydrochlorothiazide is the most famous thiazide diuretics. Losartan potassium is 2-butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1H-imidazole-5-methanol monopotassium salt, is the first member of a new class of non-peptide angiotensin II receptor antagonist. Irbesartan is 2-butyl-3-[p-(o-1H-tetrazol-5-ylphenyl) benzyl]-1, 3-diazaspiro [4.4] non-1-en-4-one. It is an orally active specific angiotensin II receptor antagonist used, as a hypotensive agent does not require biotransformation into an active form. Valsartan is N-(1-oxopentyl)-N-[[2-(1H-tetrazol-5-yl) [1, 1-biphenyl]-4-yl] methyl]-L-valine. Valsartan is a potent, highly selective, and orally active antagonist at the angiotensin II AT1- receptor. Telmisartan is 4-((2-n-propyl-4-methyl-6-(1-methylbenzimidazol-2-yl)-benzimidazol-1-yl) methyl) biphenyl-2-Carboxylic acid (Figure 1).

Experiment

Instrumentation

Balance: KERN model 870-13, Instrument Kern Balance, Supplied from Kern, Germany Model/Type/P 870-13, Serial No: 84444

High performance liquid chromatography: Consisting of instrumental

(a) AGILENT 1200 Quaternary pump.

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- (b) AGILENT 1200 Diode Array detector (DAD).
 (c) AGILENT 1200 Auto sampler (injector).
 (d) Column: ACE column dimension (250x4.6) mm particle size 5 μ Supplied from ACE.
 (e) The system equipped by Agilent chemistation PC program.
PH meter: Metrohm.
Stirrer: Fischer scientific UK.

Chemicals and reagents

All reagents used were of analytical grade or HPLC grade. Potassium dihydrogen phosphate, orthophosphoric acid and Sodium hydroxide (NaOH) were supplied by (Merck, Darmstadt, Germany), Acetonitrile and Methanol HPLC grade were supplied by (Fischer scientific, U.K.) and Distilled water.

(Note: The water used in all the experiments was obtained from Milli-RO and Milli-Q systems (Millipore, Bedford, MA).

Irbesartan, Losartan potassium, Telmisartan, Valsartan and Hydrochlorothiazide working standard powders were kindly supplied by Egyptian international pharmaceutical industries company (EIPICO) (10th Ramadan, Egypt), and were used without further purification.

Pharmaceutical preparation

X-tension plus tablets October pharma /EPCP (Egypt) contain (150 mg Irbesartan + 12.5 mg Hydrochlorothiazide) per tablet B.NO: E0230311. *Losazide* tablets (EIPICO, Egypt) contain (50 mg Losartan potassium + 12.5 mg Hydrochlorothiazide) per tablet B.NO:1002445. *Micardis plus* tablets (Boehringer Ingelheim Company, Germany) contain (80 mg Telmisartan + 12.5 mg Hydrochlorothiazide) per tablet B.NO:908577. *Disartan CO 80* tablets (Global Napi Pharmaceuticals GNP, Egypt) contain (80 mg Valsartan + 12.5 mg Hydrochlorothiazide) per tablet B.NO: 921501.

Chromatographic condition

Mobile phase: A mixture of potassium dihydrogen phosphate

buffer (pH 6.0, 0.025M) - acetonitrile (80%:20%, V/V). (Gradient elution)(Table 1).

Phosphate buffer (0.025 M) was prepared by dissolving 3.6 g potassium dihydrogen phosphate in approximately 950 ml distilled water. The pH was adjusted to 6.0 with 1 M NaOH and water was added to 1000 ml.

Column: ACE RP-C18 supplied from ACE.

Detector: was set at 220 nm.

Flow rate: 1.5 ml/min.

Column temperature: 40 C°.

Injection volume: 50 μ l.

The mobile phase was filtered through a 0.45 μ l Nylon membrane filter (Millipore, Milford, MA, USA) under vacuum and degassed by ultrasonication (Cole Palmer, Vernon Hills, USA) before usage.

Preparation of stock standard solutions

Stock standard solutions containing (1.25, 1.5, 0.5, 0.8, 0.8 mg/ml) of Hydrochlorothiazide, Irbesartan, Losartan potassium, Telmisartan, Valsartan respectively were prepared by dissolving (12.5, 150, 50, 80, 80 mg) of each in methanol in 100 ml volumetric flask respectively. It was then sonicated for 15 minutes and the final volume of solutions was made up to 100 ml with methanol to get stock standard solutions.

Preparation of calibration plot (working standard solutions)

To construct calibration plots, The stock standard solutions were diluted with the mobile phase to prepare working solutions in the concentration ranges (2.5-15, 30-180,10-60,16-96, 16-96 μ l/ml) for Hydrochlorothiazide, Irbesartan, Losartan potassium, Telmisartan and Valsartan respectively. Each solution (n=5) was injected in triplicate and chromatographed under the mentioned conditions above. Linear relationships were obtained when average drug standard peak area were plotted against the corresponding concentrations for each drug. Regression equation was computed.

Sample preparation

A composite of ten *X-tension plus* tablet, *Losazide* tablet, *Disartan CO 80 capsule* and *Micardis plus* tablet were prepared by grinding them to a fine, uniform size powder, triturated using mortar and pestle.

After calculating the average tablet weight, amounts of powder equivalent to (2.5, 150, 50, 80 and 80 mg)for Hydrochlorothiazide, Irbesartan, Losartan potassium, Telmisartan and Valsartan respectively of each type of tablets were accurately weighed and transferred separately to 100 ml volumetric flasks respectively. Solutions were sonicated for 15 min and the solutions were then filtered through 0.45 μ m Nylon membrane filters (Millipore, Milford, MA, USA). Aliquots of appropriate volume (10 ml) were transferred to 100 ml calibrated flasks

Time (min)	Buffer %	Acetonitrile %
0	20	80
5	35	65
15	35	65
16	20	80
17	20	80

Table1: Time table of validated gradient method.

and diluted to volume with mobile phase to furnish the mentioned concentration above. The diluted solutions were analyzed under optimized chromatographic conditions and chromatogram is depicted in (Figure 2).

Method Validation

Specificity

Specificity of the method was evaluated by assessing whether excipients present in the pharmaceutical formulations interfered with the analysis or not [21]. A placebo for each tablet was prepared by mixing the respective excipients and solutions were prepared by following the procedure described in the section of sample preparation. The commonly used tablet excipients did not interfere with the method. The diluent chromatogram in (Figure 3) shows that the tablet diluent has negligible contribution after the void volume at the method detection wavelength of 220 nm.

The method were also evaluated by assessing whether degradation products present in the pharmaceutical formulations interfered with the analysis, obtained from stress studies involving acid, base, peroxide and heat as well as analysis of samples stored under ICH stability conditions. Chromatograms are also shown in (Figure 3) to demonstrate method specificity.

Linearity and range

The linearity of the method was evaluated by analyzing different concentration of the drugs. According to ICH recommendations [21] at least five concentrations must be used. In this study five Concentrations were chosen, in the ranges (2.5-15, 30-180, 10-60, 16-96, and 16-96 µl/ml) corresponding levels of 20-120% w/w of the nominal analytical concentration for Hydrochlorothiazide, Irbesartan, Losartan potassium, Telmisartan and Valsartan respectively. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equations were $\{Y = 223.09 X + 4.000 (r = 0.9999), Y = 122.54 X + 0.8806 (r = 1.0), Y = 138.95 X + 9.9936 (r = 1.0), Y = 197.86 X + 120.99 (r = 0.9997), Y = 122.07 X + 33.45 (r = 1.0)\}$ for Hydrochlorothiazide, Irbesartan, Losartan potassium, Telmisartan and Valsartan respectively. Where Y is the peak area of standard solution and X is the drug concentration.

Precision

The precision of the assay was investigated by measurement of both repeatability and Intermediate precision.

Figure 2: Typical HPLC chromatograms obtained from 50 µl injections of Hydrochlorothiazide (3.86 min.), Valsartan (5.8 min.), Losartan potassium (7.92 min.), Irbesartan (9.64 min.) and Telmisartan (13.81 min.) respectively under optimized chromatographic conditions.

Figure 3: Typical HPLC chromatograms obtained from 50 µl injections of a- tablet placebo under optimized chromatographic conditions b- Irbesartan, Losartan potassium and Valsartan respectively obtained from stress studies involving acid, base and heat as well as analysis of samples stored under ICH stability conditions under optimized chromatographic conditions.

Repeatability: Repeatability was investigated by injecting 6 determinations at 100% of the test concentration percentage RSD were calculated in Table 2.

Intermediate precision: In the inter-day studies, standard and sample solutions prepared as described above, were analyzed in triplicate on three consecutive days at 100% of the test concentration and percentage RSD were calculated (Table 3).

Accuracy

Accuracy was assessed using 9 determinations over 3 concentration levels covering the specified range (80,100 and 120%). Accuracy was reported as percent recovery by the assay of known added amount of analyte in the sample (Table 4).

Limits of detection and Limits of quantitation

According to the ICH recommendations [21], determination of limits of detection and quantitation was based on the standard deviation of the y-intercepts of regression lines (n=3) and the slope of the calibration plots (Table 5).

Robustness

Robustness of an analytical procedure is a measure of its capacity

to remain unaffected by small variations in method parameters and provides an indication of its reliability during normal usage [21]. Robustness was tested by studying the effect of changing mobile phase pH by ± 0.1 , the amount of acetonitrile in the mobile phase by $\pm 2\%$, temperature $\pm 2^\circ\text{C}$, different column and flow rate $\pm 0.05\text{ ml/min}$ had no significant effect on the chromatographic resolution of the method.

Stability of analytical solution: Also as part of evaluation of robustness, solution stability was evaluated by monitoring the peak area response. Standard stock solutions in methanol were analyzed right after its preparation 1, 2 and 3 days after at 5°C and for a day at room temperature. The change in standard solution peak area response over 3 days was (0.68, 0.59, 0.64, 0.32 and 0.77%) for Hydrochlorothiazide, Irbesartan, Losartan potassium, Telmisartan and Valsartan respectively. Their solutions were found to be stable for 3 days at 5°C and for a day at room temperature at least.

Application on pharmaceutical Preparation

The proposed methods were successfully used to determine

Irbesartan, Losartan potassium, Telmisartan and Valsartan respectively in their dosage forms in presence of Hydrochlorothiazide e.g. *X-tension plus* tablets, *Losazide* tablets, *Micardis plus* tablets, *Disartan co* tablets respectively. Five replicate determinations were performed. Satisfactory results were obtained for each compound in good agreement with label claims (Table 6 and 7). The results obtained were compared statistically with those from published methods [5,11,13,16] by using Student's t-test and the variance ratio F-test. The results showed that the t and F values were smaller than the critical values. So, there were no significant differences between the results obtained from this method and published methods (Table 8).

Results and Discussion

Optimization of chromatographic condition

Several trials were carried out to obtain simple, rapid simultaneous determination for four angiotensin receptor antagonists e.g. Losartan potassium, Irbesartan, Telmisartan and Valsartan in presence of Hydrochlorothiazide by high performance liquid chromatography

Drug name	Average $\mu\text{g/ml}$	Average %	RSD
Hydrochlorothiazide	12.60	100.80	0.20%
Irbesartan	150.68	100.45	0.26%
Losartan potassium	50.24	100.48	0.25%
Telmisartan	80.52	100.65	0.21%
Valsartan	80.36	100.45	0.26%

Table 2: Repeatability of Hydrochlorothiazide, Irbesartan, Losartan potassium, Telmisartan and Valsartan respectively.

Drug name	1 st day $\mu\text{g/ml}$	2 nd day $\mu\text{g/ml}$	3 rd day $\mu\text{g/ml}$	pooled average	pooled average %	RSD
Hydrochlorothiazide	12.60	12.41	12.58	12.52	100.22	0.80%
Irbesartan	150.68	149.51	148.59	149.59	99.73	0.70%
Losartan potassium	50.23	49.92	49.48	49.88	99.78	0.75%
Telmisartan	80.52	80.00	80.18	80.23	100.29	0.32%
Valsartan	80.36	79.86	78.91	79.71	99.60	0.92%

Table 3: Intermediate precision of Hydrochlorothiazide, Irbesartan, Losartan potassium, Telmisartan and Valsartan respectively.

Drug name	Recovery at 80% conc. (%)	Recovery at 100% conc. (%)	Recovery at 120% conc. (%)	Average Recovery (%)	RSD
Hydrochlorothiazide	100.50	100.84	100.22	100.52	0.31%
Irbesartan	100.18	100.62	100.37	100.39	0.22%
Losartan potassium	100.23	101.66	100.36	100.75	0.78%
Telmisartan	101.48	100.80	99.58	100.62	0.96%
Valsartan	100.63	100.63	100.21	100.49	0.24%

Table 4: Recovery results for standard solution plus excipients for Hydrochlorothiazide, Irbesartan, Losartan potassium, Telmisartan and Valsartan respectively.

Item	HCTZ	Irbesartan	Losartan	Telmisartan	Valsartan
Linear range ($\mu\text{g/ml}$)	2.5-15	30-180	10-60	16-96	16-96
Detection limit ($\mu\text{g/ml}$)	0.04	0.14	0.08	0.03	0.04
Quantitation limit ($\mu\text{g/ml}$)	0.11	0.44	0.24	0.10	0.11
Regression data					
N	5	5	5	5	5
Slope (b)	223.09	122.54	138.95	197.86	33.45
Standard deviation of the slope	1.11	0.26	0.20	0.20	0.20
Intercept (a)	4.00	0.88	9.99	120.99	122.07
Standard deviation of the intercept	3.71	16.4	5.91	8.24	7.14
Correlation coefficient R^2	0.9999	1.0	1.0	0.9997	1.0
Standard error of regression	9.85	18.46	11.19	125.95	26.75

($Y = a + bC$, where C is the concentration of the compound ($\mu\text{g/ml}$) and Y is the drug peak area)

Table 5: Calibration data was resulted from method validation of Hydrochlorothiazide (HCTZ), Irbesartan, Losartan potassium, Telmisartan and Valsartan respectively.

Product name	X-tension plus tablets		Losazide tablets		Micardis plus tablets		Disartan co tablets	
Drug name	IRB (%)	HCTZ (%)	LOS (%)	HCTZ (%)	TEL (%)	HCTZ (%)	VAL (%)	HCTZ (%)
Test 1	97.43	96.25	100.3	99.51	99.87	99.47	99.7	101.4
Test 2	99.22	97.53	101	99.83	100.1	100.3	100.2	100.3
Test 3	99.34	97.63	99.35	100.2	99.66	99.49	100.2	102.1
Test 4	100.6	98.72	100.4	99.69	99.93	100.1	100.4	100.5
Test 5	100.7	98.72	100.7	100.2	99.95	100.1	97.54	97.04
SD	1.33	1.02	0.62	0.31	0.16	0.39	1.19	1.94
Average	99.46	97.77	100.35	99.88	99.90	99.89	99.61	100.27
R.S.D	1.32	1.05	0.61	0.30	0.16	0.39	1.19	1.94

Table 6: Results from determination of Irbesartan (IRB), Losartan potassium (LOS), Telmisartan (TEL) and Valsartan (VAL) in presence Hydrochlorothiazide (HCTZ) respectively in their dosage forms by proposed method.

Product name	X-tension plus tablets		Losazide tablets		Micardis plus tablets		Disartan co tablets	
Drug name	IRB (%)	HCTZ (%)	LOS (%)	HCTZ (%)	TEL (%)	HCTZ (%)	VAL (%)	HCTZ (%)
Test 1	97.04	97.16	99.71	100.8	99.86	99.9	97.55	97.76
Test 2	99.44	97.88	99.98	99.65	99.8	99.88	99.93	101.9
Test 3	99.49	97.38	99.95	99.71	100.1	99.94	100.6	101
Test 4	99.28	99.22	100.8	100.7	99.81	100.3	100.2	102.2
Test 5	99.67	99.11	100.5	100.8	99.32	100.1	100.6	101.2
SD	1.1	0.96	0.45	0.60	0.28	0.18	1.28	1.78
Average	98.98	98.15	100.19	100.33	99.78	100.02	99.77	100.81
R.S.D	1.10	0.98	0.45	0.59	0.28	0.18	1.28	1.76
Method number	5		16		11		13	

Table7: Results from determination of Irbesartan (IRB), Losartan potassium (LOS), Telmisartan (TEL) and Valsartan (VAL) in presence Hydrochlorothiazide (HCTZ) respectively in their dosage forms by reported (published) method.

Drug name		Recovery $\pm$ SD		Calculated t- values	Calculated F- values
		Proposed methods	Reference method		
X-tension plus tablets	IRB (%)	99.46 $\pm$ 1.33	98.98 $\pm$ 1.10	1.54	1.46
	HCTZ (%)	97.77 $\pm$ 1.02	98.15 $\pm$ 0.96	2.04	1.13
Losazide tablets	LOS (%)	100.35 $\pm$ 0.62	100.19 $\pm$ 0.45	0.54	1.94
	HCTZ (%)	99.88 $\pm$ 0.31	100.33 $\pm$ 0.60	1.31	0.27
Micardis plus tablets	TEL (%)	99.90 $\pm$ 0.16	99.77 $\pm$ 0.28	0.71	0.32
	HCTZ (%)	99.89 $\pm$ 0.39	100.02 $\pm$ 0.18	0.82	4.74
Disartan co tablets	VAL (%)	99.61 $\pm$ 1.19	99.77 $\pm$ 1.28	0.20	0.86
	HCTZ (%)	100.27 $\pm$ 1.94	100.81 $\pm$ 1.78	0.41	1.20

(Where the Tabulated t-values and F -ratios at p = 0.05 are 2.57 and 5.05)

Table 8: Statistical comparison of the proposed and published methods for determination of Irbesartan (IRB), Losartan potassium (LOS), Telmisartan (TEL) and Valsartan (VAL) in presence Hydrochlorothiazide (HCTZ) respectively in their dosage forms by reported method (T- student test) and (F –test for variance).

(HPLC) method with low cost, simple mobile phase to be safe and more available accessories e.g. detectors and column. Hypersil BDS C-18 (25cm) columns were tested for separation using a mobile phase consisted of potassium dihydrogen phosphate buffer pH=3.5 and acetonitrile in different ratio (Figure 4). Different buffers have been also used for good separation between four drugs especially between Valsartan and Irbesartan e.g. ammonium acetate 0.02M and citrate buffer 0.02M at the same pH= 3.5 adjusting pH by glacial acetic acid and citric acid respectively (Figure 5).

Controlling mobile phase pH has important role when analyzing ionizable compounds (Valsartan and telmisartan) by reversed phase (RP) HPLC, it can be recognized and easily understood. In reversed phase HPLC, the retention of analytes is related to their hydrophobicity. The more hydrophobic the analyte, the longer it is retained. When an analyte is ionized, it becomes less hydrophobic and therefore, its retention decreases. Acids lose a proton and become ionized and carry

a negative charge when pH increases (at pH's above the analyte's pKa) so behaves as an extremely polar molecule and bases gain a proton and become ionized when pH decreases (Figure 6) therefore, when separating mixtures containing acids and/or bases by reversed phase HPLC, it is necessary to control the pH of the mobile phase using an appropriate buffer in order to achieve reproducible results [22,23]. Addition of a phosphate buffer at higher pH eliminated the broad tailing peaks and created rugged conditions suitable for successful assay. In this study, Losartan potassium is ionized compound, Irbesartan is non ionized compound and Valsartan and Telmisartan may be turn to ionizable compound by changing pH of mobile phase due to presence of carboxylic group (-COOH) in their chemical structure.(Remember: There is a problem in separation between Valsartan and Irbesartan). Trials were carried out under different pH of phosphate buffer on a Hypersil BDS C-18 (25cm) column. At pH=2.5 showed poor separation and long time 17.0 minutes, at pH=4.5 consumed longer time about

22.0 minutes and at pH=6.0 shows good resolution, good separation and shorter time (Figure 7).

As pH=6.0 greater than pKa of Valsartan by difference equals to 1.1 which enables (-COOH) to give carboxylate anion (-COO⁻) carrying negative charge so elute rapidly than others. Telmisartan also elute more rapidly than other pH.

The concentration of the mobile phase buffer usually has little effect on retention in reversed phase HPLC, just as long as the buffer concentration is high enough to control pH. A buffer concentration in the range of 25 to 50 mM is adequate for reversed phase applications [23].

Finally, it was found that ACE C-18 column give more good separation and band spacing more than Hypersil BDS C-18 column and sharper peak shape. But for analysis of ARA-II drugs in presence of Hydrochlorothiazide, a new problem is appeared which is interfering between Valsartan and Hydrochlorothiazide. So gradient elution was tried to solve it as in (Figure 2).

Conclusion

A simple, accurate, precise, robust and reliable LC method has been established for simultaneous determination for Irbesartan, Losartan

a- Ammonium acetate buffer:

b- Citrate buffer

Figure 5: HPLC chromatogram of Hypersil BDS C-18 (25cm) Column and mobile phase consisted of (A) acetonitrile and (B) buffer pH=3.5 in ratio 35:65% where a- ammonium acetate 0.02M b- citrate buffer 0.02M for trials to separate Losartan potassium(LOS), Irbesartan (IRB), Valsartan (VAL) and Telmisartan (TELM) respectively.

As acids lose a proton and become ionized (with increasing pH), their retention decreases. As bases gain a proton and become ionized (with decreasing pH) their retention decreases.

Figure 6: Effect of pH on the retention of acids and bases in reversed phase HPLC.

potassium, Telmisartan, Valsartan in presence of Hydrochlorothiazide in their formulations. The method has several advantages:

1. The first is using HPLC-UV which is the most available instrument in pharmaceutical analysis in companies due to its low cost in comparison with methods utilized electrophoresis, HPLC coupling with fluorimetric detection or mass spectroscopy.
2. High sensitive method has LOD range (0.04-0.14) µg/ml and LOQ range (0.1-0.44) µg/ml.
3. It is suitable for analysis of antihypertensive agents in their formulations in a single run, in contrast with previous methods. This makes the method suitable for routine analysis in quality-control laboratories. Other merits are rapid analysis, a simple mobile phase, simple sample preparation, does not use polluting reagents.
4. Illustration of steps of developing validated HPLC method

depending on physicochemical properties of drugs especially solubility and pKa of drugs.

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