IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 3 Issue 7, July 2016
ISSN (Online) 2348 – 7968 | Impact Factor (2015) - 4.332
www.ijiset.com

Development and Validation Of A Simple Isocratic HPLC-UV Method For Simultaneous Determination Of Amlodipine Besylate And Losartan Potassium In Their Combined Tablet Dosage Forms

Babiker Y. Abdulkhair [a*], Mohamad A. Abdulaziz[a]

[a] Chemistry Departemt, College of Science, Sudan University For Science and Technology, Khartoum, Sudan.

Abstract:

A rapid and simple isocratic HPLC-UV method was validated for the simultaneous determination of amlodipine besylate (AML) and losartan potassium (LOS) in combined tablet dosage forms. for simplicity an isocratic chromatographic conditions selected, an excellent separation was obtained using phenyl-Hexyl column (150 mm × 4.6 mm, 3µm), formic acid solution (1% v/v) : methanol in 25:75 ratio as mobile phase with 0.8 ml/min flow rate, 20 µl injection volume, 25^{o}C (ambient temperature) as column temperature and 275 nm for simultaneous detection of both components. As its obvious here the conditions optimized were extremely simple and general so this method could be applied with non advanced HPLC systems. The retention times was 3.5 min and 4.4 min for AML and LOS respectively, the linearity ranges were 0.57-32µg/ml AML and 4.8-320µg/ml LOS. The R^2 was 1.000 for both active ingredients. Method robustness was tested under nine different conditions, the average of the nine assays for AML and LOS was 99.65% and 99.76 respectively and the RSD of the nine assays was 0.26% and 0.18 respectively. This simple isocratic HPLC method is repeatable, reproducible and robust enough to be used for research and quality control purposes for this combination.

Keywords: Isocratic method ; HPLC-UV; Method validation; Amlodipine besylate; Losartan potassium.

1. Introduction

Hypertension is one of the most common diseases affecting people, many drugs were used hypertension control and it had been found that multi component drugs are more effective than single drugs [1]. Many combinations were used as a single daily pill to achieve a more positive effects [2]. AML shown in Figure 1, is a calcium channel blockers that acts by relaxing smooth muscle in the arterial wall, its efficient in patients with artery disease, heart failure, exerting no unfavorable effects on carbohydrate and lipid metabolism. [3,4]. LOS shown in Figure 2, is used for chronic heart failure, diabetic and hypertension [5]. A variety of methods were reported for determination of these active ingredients either single or in combination with others, such as micro emulsion liquid chromatography [6] HPTLC [7,8], LC-Mass [2,9,10]. LC-UV methods had been developed for assay of this combination [11-14] and for other combinations that contains one or more of the three active ingredients[15-17]. LC-Mass is the advanced system now a days but because of its expense of buy and use its rarely used in quality control Laboratories, and because LC-UV is more available there so that anew isocratic HPLC-UV method was optimized to be applied with a minimum requirements of an HPLC system (one pump), UV detection at fixed wavelength, ambient temperature (without column oven) the

buffer solution is easy to prepare (1% formic acid solution without pH adjustment). Moreover, the method is economic (flow rate 0.8 ml/min for six min per injection) .This method had been tested for possible changes in flow rate, mobile phase composition, column temperature and detection wavelength. The pH adjustment is not required in the preparation of mobile phase since 1% formic acid is 0.2624 M ($2624*10^{-4}$ mole) solution while the ionization constant is just $1.8*10^{-4}$ mole so the pH of the buffer solution stay unaffected by slight error in preparation by 0.1%, i.e. 0.9% or 1.1%. this method with all these facilities pass all validation tests according to International conference of Harmonization (ICH)[18], to the best of our knowledge, no simpler isocratic method had been reported for an assay of this mixture in tablet dosage forms.

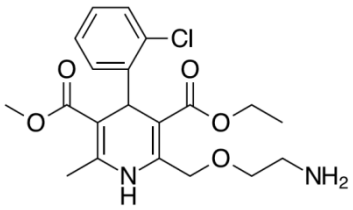

Figure 1 Chemical structure of AML

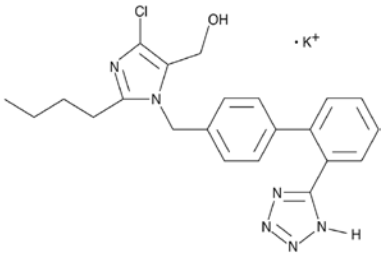

Figure 2 Chemical structure of LOS

2.1 Materials

Working standards AML, LOS and excipients were supplied from Blue Nile Pharmaceuticals. Acetonitrile and formic acid were HPLC grade (Scharlau Spain). HPLC grade water was used.

2.2. Instrumentation

The HPLC-UV system consisted of a Shimadzu LC-2010A HT series apparatus (Shimadzu Corporation, Tokyo, Japan) with a quaternary pump, online degasser, UV detector, column oven and auto sampler. This system was connected to a computer loaded with LC-Solutions software. A Phenyl-Hexyl column (150 mm x 4.6 mm, I.D. 3 µm) was selected. DAD, Shimadzu Corporation, Tokyo - Japan, Prominence, Sr. No. L20154807000AE was applied for selection of proper detection wavelength.

2.3. Methods

2.3.1. Standard stock solution

0.05g amlodipine besylate and 0.5g losartan potassium were weighed accurately and transferred quantitatively to 50 ml volumetric flask. The flask half filled with mobile phase and sonicated for 10 minutes, cooled to room temperature, then the volume was completed to the mark with mobile phase.

2.3.2. Standard solution

Subsequent dilutions were made from the standard stock solution with mobile phase to produce 20µg/ml amlodipine besylate and 200µg/ml losartan potassium.

2.3.3. Assay preparation

Twenty tablets were weighed and grinded, average weight of tablets was transferred to 50ml volumetric flask, the flask half filled with mobile phase, then sonicated for 10 minutes, cooled to room temperature and completed to the mark with the mobile phase, Subsequent dilutions were made in mobile phase in same manner of the standard to achieve target concentration.

3. Results

3.1. Method optimization

The chromatographic conditions were optimized to satisfy system suitability parameters for the two active ingredients, detection wavelength was selected using photodiode array detector, mobile phase composition, flow rate, column and column temperature were altered until the resulting resolution, tailing factor, theoretical plates and relative standard deviation for area of six injections were within the acceptance limits according to ICH. The conditions affecting these parameters. The optimized chromatographic condition were isocratic elution with a flow rate of 0.8 ml/min for a mobile phase composed of 1%v/v formic acid solution : methanol in 25:75 respectively, injection volume 20 µl, phenyl-hexyl column (150 mm x 4.6 mm, 3 µm) was suitable for separation, 260 nm was selected for detection of the two components simultaneously and the method was optimized at 25oC.

3.1.1. System suitability

The system suitability test is an integral part of the analytical method. For this, a mixed standard solution (target concentration) was injected six times. Parameters such as RSD% for the peak area, retention time, resolution and theoretical plates of the peaks were calculated. test results were shown in Table 1and Table 2, for AML and LOS respectively.

Table 1 System suitability parameters for AML

	Retention time	Area	Theoretical plates	Tailing factor	Resolution
STD 1	3.36	305882	5677	1.65	6.061
STD 2	3.306	305538	5577	1.633	6.081
STD 3	3.288	306284	5550	1.644	6.103
STD 4	3.28	306195	5468	1.64	6.057
STD 5	3.284	306432	5490	1.658	6.061
STD 6	2.301	307485	5444	1.651	6.037
Average	3.1365	306302.7	5534.333333	1.646	6.066666667
STDEV	0.41037629	661.552	85.95968047	0.008876936	0.022642144
RSD	13.0838926	**0.21598**	1.553207501	0.539303549	0.373222147

Table 2 System suitability parameters for LOS

	Retention time	Area	Theoretical plates	Tailing factor	Resolution
STD 1	4.46	4012771	9352	1.361	6.061
STD 2	4.402	4018013	9198	1.36	6.081
STD 3	4.382	4020623	9255	1.361	6.103
STD 4	4.375	4023237	9047	1.369	6.057
STD 5	4.381	4023730	8997	1.371	6.061
STD 6	4.392	4023393	9065	1.371	6.037
Average	4.39866667	4020295	9152.333333	1.3655	6.066666667
STDEV	4.38844444	4289.006	138.0893431	0.005357238	0.022642144
RSD	0.24455716	0.106684	1.508788393	0.392327945	0.373222147

3.1.2. Selectivity

Mixed standard solution, sample and placebo solutions were prepared, each solution was injected and chromatograms were Figure 3 . The were prepared by taking the weight of placebo equivalent to its weight in the test preparation. Based on the chromatograms of the sample Figure 4 and placebo Figure 5 , the placebo solutions showed no peaks at the retention time of the AML and LOS peaks. This indicates that the excipients used in the formulation did not interfere in the estimation of the active ingredients in the tablets. Also, based on Figure.3 and Figure 4, the system suitability parameters in the sample chromatogram were almost equal to those of the standard chromatogram (i.e. the excipients in the sample did not retard separation).

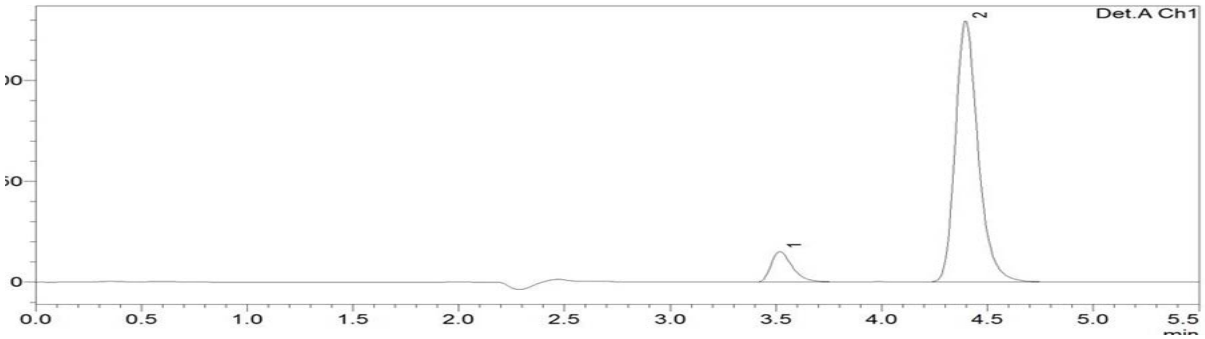

Fig 3 Chromatogram of mixed standard solution at the optimized conditions

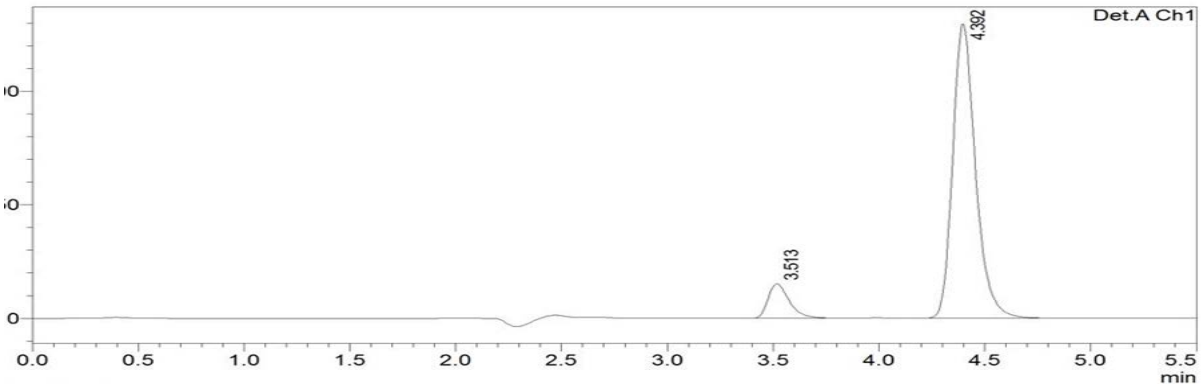

Figure 4 Chromatogram of sample solution at the optimized conditions

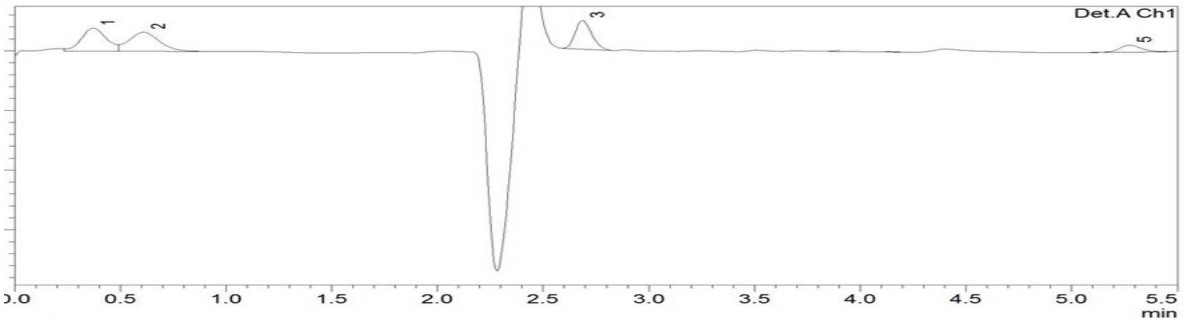

Figure 5 Chromatogram of placebo solution at the optimized conditions

3.1.3. Linearity

Seven concentrations were prepared, in the range 40% to 160% of target analyte concentrations; typically the solutions 8, 12, 16, 20 ,24 ,28 and 32µg/ml AML and 80, 120, 160, 200, 240, 280 and 320 µg/ml LOS. Solutions were prepared in the mobile phase as mixed standards. Each mixed standard solution was injected in triplicate and the mean value of the peak area was used for the calibration curve. The calibration graphs were obtained using XL-STAT 2015 program. The linear regression plots for AML and LOS shown in figure 6 and figure 7 respectively, regression equations were

$$\text{Area} = -1038.33+15392.024*\mu g/ml$$

$$\text{Area} = 13330.33+20045.62*\mu g/ml$$

respectively. The regression coefficient values (R^2) were found to be 1.000 for both analytes, indicating an excellent linear relationship for this method.

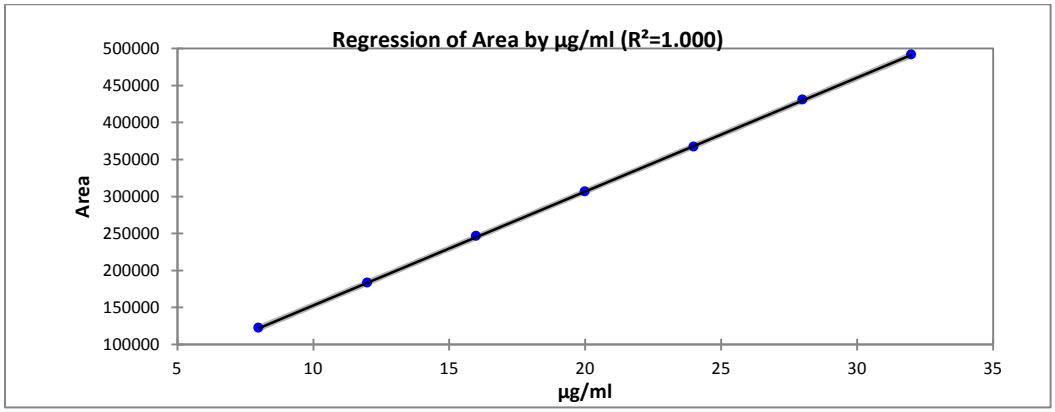

Figure 6 XL- STAT 2015 plot of conc. µg/ml versus peak area for amlodipine besylate

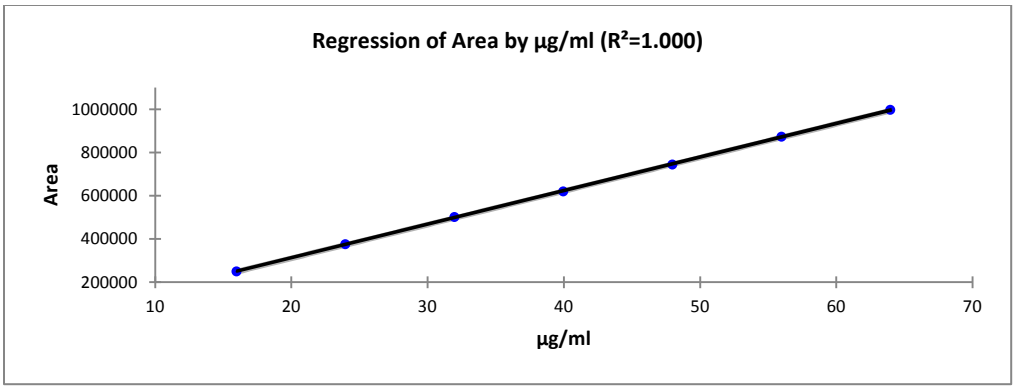

Figure 7 XL- STAT 2015 plot of (µg/ml) Vs (peak area) for losartan potassium

3.1.4. Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated from linearity data according to ICH: LOD = 3.3* (SD/S) and LOQ = 10 * (SD/S). The LOD was found to be 0.19µg/ml, µg/ml and 1.6 µg/ml for AML and LOS respectively, while the LOQ values were 0.57 µg/ml and 4.8 µg/ml, respectively.

3.1.5. Accuracy

Seven 100ml volumetric flasks were labeled, a placebo equivalent to tablet's weight was transferred to each flask. A volume of standard stock solution required to produce 40%, 60%, 80%, 100%, 120%, 140% and 160% tablet's content AML and LOS were added each to different 100ml volumetric flask. The flasks were half filled with mobile phase, sonicated for 10 minutes, cooled to room temperature, then completed to the mark with the same solvent. Subsequent dilutions were made with mobile phase in same manner of the standard preparation. Each solution was injected three times, Table 3, and Table 4 predict the recovery percentages of AML and LOS respectively, all the obtained results were within the permissible limits according to ICH guidelines [20].

Table 3 accuracy results for AML

Standard		Samples							
No.	AML	% content→	40	60	80	100	120	140	160
STD1	305882	Trial 1	122376	183133	245821	305628	367451	428743	489622
SDT2	305538	Trial 1	122420	184144	245852	305314	366546	430400	488167
STD3	306284	Trial 1	122551	183721	245949	305289	367485	429390	488890
STD4	306195	Avg.	**122449**	**183666**	**245874**	**305410**	**367161**	**429511**	**488893**
STD5	306432	STDEV	91.032961	507.739106	66.7757441	188.9189	532.5883	835.1006	727.50464
STD6	307485	RSD	0.0743436	0.27644698	0.02715852	0.061857	0.145056	0.194431	0.1488065
Avg.	**306302.7**	RECOVERY	**39.97647**	**59.96226**	**80.271583**	**99.7087**	**119.869**	**140.224**	**159.6111**
STDEV	661.55201	RECOV %	**99.94118**	**99.937099**	**100.33948**	**99.7087**	**99.8905**	**100.16**	**99.75693**
RSD	**0.21598**								

Table 4 accuracy results for LOS

Standard		Samples							
No.	LOS	% content→	40	60	80	100	120	140	160
STD1	4012771	Trial 1	1612979	2412016	3235948	4017636	4782932	5583003	6407021
SDT2	4018013	Trial 1	1613082	2423934	3236321	4018188	4795053	5595200	6398694
STD3	4020623	Trial 1	1613918	2416244	3235999	4018278	4786685	5594811	6393788
STD4	4023237	Avg.	**1613326**	**2417398**	**3236089**	**4018034**	**4788223**	**5591005**	**6399834**
STD5	4023730	STDEV	514.9799	6042.2237	202.243	347.6032	6205.2	6932.376	6689.79
STD6	4023393	RSD	0.031920	0.2499474	0.00625	0.008651	0.129593	0.123992	0.104530
Avg.	**4020295**	RECOVERY	**40.12956**	**60.129874**	**80.4938**	**99.9438**	**119.101**	**139.07**	**159.1882**
STDEV	4289.006	RECOV %	**100.3238**	**100.21645**	**100.62**	**99.94377**	**99.25109**	**99.33537**	**99.49262**
RSD	**0.106684**								

3.1.6. Precision

- **Intraday precision**

Three 100 ml volumetric flasks were labeled, a placebo equivalent to one tablet was transferred to each flask. The volume of the standard stock solution required to produce 80%, 100% and 120% of the tablet content of AML and LOS was added to the placebo. The flasks were half-filled with the mobile phase, sonicated for 10 minutes, cooled to room temperature and completed to the mark with the same solvent. Subsequent dilutions were made with the mobile phase in the same manner of standard preparation. The assay was performed for these solutions five times in one day; each solution was injected three times for each assay, averages of triplicates were used for recovery percentage calculation, the average of the five recovery percentages for 80%, 100% and 120% AML were 100.28%, 99.72% and 99.92% respectively and 100.61%, 99.98% and 99.18% respectively for LOS, all these values were within permissible limits (100 $\pm$ 2.5)%. The relative standard deviation (RSD%) for each five assays of 80%, 100% and 120% for AML they were 0.04%, 0.08% and 0.12%, while they were 0.03, 0.04 and 0.03 for LOS, all RSD values were within the permissible limits (RSD% $\leq$ 2.0).The detailed results were shown in table 5.

Table 5 intraday precision results AML and LOS

	AML			LOS		
% content→	**80%**	**100%**	**120%**	**80%**	**100%**	**120%**
Assay 1	100.3087	99.86517	100.0773	100.6555	100.0554	99.22445
Assay 2	100.3128	99.68539	100.0075	100.6192	99.97362	99.19047
Assay 3	100.2983	99.69583	99.86038	100.6007	99.96329	99.18351
Assay 4	100.2644	99.65481	99.84115	100.5837	99.93528	99.1588
Assay 5	100.2184	99.68561	99.80515	100.5912	99.97034	99.15741
Avg.	**100.2805**	**99.71736**	**99.91829**	**100.6101**	**99.97958**	**99.18293**
STDEV	0.039597	0.084044	0.117626	0.028672	0.044988	0.027455
RSD	**0.039486**	**0.084282**	**0.117722**	**0.028498**	**0.044997**	**0.027682**

- **Intraday precision**

Three 100 ml volumetric flasks were labeled, a placebo equivalent to one tablet was transferred to each flask. The volume of the standard stock solution required to produce 80%, 100% and 120% of the tablet content of AML and LOS was added to the placebo. The flasks were half-filled with the mobile phase, sonicated for 10 minutes, cooled to room temperature and completed to the mark with the same solvent. Subsequent dilutions were made with the mobile phase in the same manner of standard preparation. The assay was performed for these solutions three times each time on separate day; each solution was injected three times for each assay, averages of triplicates were used for recovery percentage calculation, the average of the three recovery percentages for 80%, 100% and 120% AML were 100.18%, 99.71% and 100.00% respectively and 99.98%, 100.45% and 99.03% respectively for LOS, all these values were within permissible limits (100 $\pm$ 2.5)%. The relative standard deviation (RSD%) for each three assays of 80%, 100% and 120%, for AML they were 0.33%, 0.14% and 0.07%, while they were 0.06, 0.17 and 0.17 for LOS, all RSD values were within the permissible limits (RSD% $\leq$ 2.0).The detailed results were shown in table 6.

Table 6 interday precision results AML and LOS

	AML			**LOS**		
% content→	**80%**	**100%**	**120%**	**80%**	**100%**	**120%**
Day 1	100.3087	99.86517	100.0773	100.0554	100.6555	99.22445
Day 2	100.4279	99.65318	99.99844	99.95189	100.3829	98.93367
Day 3	99.80591	99.6041	99.93206	99.92452	100.3343	98.94723
Avg.	100.1809	99.70748	100.0026	99.97727	100.4576	99.03512
STDEV	0.330138	0.138748	0.072711	0.069024	0.173138	0.164111
RSD	0.329542	0.139155	0.072709	0.069039	0.172349	0.16571

3.1.7 Robustness

The robustness was assessed by evaluating the effect of small but deliberate variations in the chromatographic conditions. An assay was performed for target concentration under the following nine conditions: optimum conditions, column temperature plus 5^{o}C, column temperature minus 5^{o}C, increasing organic solvent 5% in mobile phase, decreasing organic solvent 5% in mobile phase, increasing flow rate 5%, decreasing flow rate 5%, detection wavelength plus 3 nm and detection wavelength minus 3nm. The average of these assays under these different conditions for AML and LOS were 99.65 and 99.76 respectively, the relative standard deviations for AML and LOS were 0.26, 0.36 and 0.18 respectively, the detailed results were shown in Table 7.

Table 7 results of method robustness for AML and LOS

	Recovery	
% content→	AML	LOS
Optimized conditions	99.70867595	99.94377278
More 5 degree Celsius	99.89408809	99.79900504
less 5 degree Celsius	99.78218499	99.75562597
5% More flow rate	99.35702219	99.58700892
5% less flow rate	99.14427436	99.37924743

5% more Organic solvent	99.98635399	99.97884431
5% less Organic solvent	99.68990635	99.84946469
More 3 nm	99.54165434	99.7256177
Less 3 nm	99.56330193	99.78429709
Avg.	**99.65178656**	**99.75613845**
STDEV	**0.262766244**	**0.182937003**
RSD	**0.263684428**	**0.183384207**

3.3 Application of method for assay of real sample

The validated method was applied for analysis of combined tablets from local market of Riyadh -KSA. The standard solution and sample were prepared as described in section 2.3. The assay results were 100.2% AML and 100.17% LOS, detailed results were shown in Table 8

Table 10 results of method application for real samples

	Standard			Samples	
	AML	LOS		AML	LOS
STD1	146064	2947909	Assay 1	147664	2952944
SDT2	146875	2948752	Assay 2	147664	2952944
STD3	147677	2948303	Assay 3	147776	2948897
STD4	147013	2948774	avg	147701.3	2953608
STD5	147166	2948493	STDEV	64.66323	2336.5365
STD6	148323	2948280	RSD	0.04378	0.07910787
avg	147410.8	2948520.	Percentage	**100.20**	**100.17**
STDEV	593.343239	236.5149			
RSD	0.40251001	0.008021			

References

[1] Jafarzadeh Esfehani R, Mahmoodi Gharai A, Jafarzadeh Esfehani A, Rezaie Kalat A, Abbasi F., Jalalyazdi A.A Comparative Study of the Management of Stage 2 hypertension by Combined therapy with Losartan, Amlodipine and Hydrochlorothiazide. Int Cardiovasc Res J. 2012;6(3) : 79-83.

[2] Shankar Ganesh Gadepalli, Pragney Deme, Madhusudana Kuncha, Ramakrishna Sistla, Journal of Pharmaceutical Analysis 2014;4(6):399–406.

[3] Levine, C.B., Fahrbach, K.R., Frame, D., Connelly, J.E., Estok, R.P., Stone, L.R., Ludensky,

V.: Effect of amlodipine on systolic blood pressure. Clin Ther, (2003), (1), 25-35.

[4] Steffen H-M, Amlodipine - a third generation dihydropyridine calcium antagonist, Journal of Clinical and Basic Cardiology 1999; 2 (1), 45-52.

[5] Gemma Currie, Herjit Sidhu, Miles Fisher, Gerry McKay, Practical diabetes vol. 30 No. 2, Copyright © 2013 JOHN WILEY & SONS, pp. 91–93.

[6] Mohammed E. A. Hammouda, Mohamed A. Abu El-Enin, Dina T. El-Sherbiny, Dalia R. El-Wasseef and Saadia M. El-Ashry; Simultaneous Determination of Enalapril and Hydrochlorothiazide in Pharmaceutical Preparations Using Microemulsion Liquid Chromatography; Journal of Chromatographic Science 2015;53:90–96

[7] Karunanidhi Santhana Lakshmi and Sivasubramanian Lakshmi, Simultaneous Analysis of Losartan Potassium, Amlodipine Besylate, and Hydrochlorothiazide in Bulk and in Tablets by

High-Performance Thin Layer Chromatography with UV-Absorption Densitometry,

Journal of Analytical Methods in Chemistry 2012, Article ID 108281, 5 pages.

[8] Ibrahim A. Naguib, Eglal A. Abdelaleem, Hala E. Zaazaa and Mohammed E. Draz; Simultaneous Determination of Hydrochlorothiazide and Benazepril Hydrochloride

or Amiloride Hydrochloride in Presence of Hydrochlorothiazide Impurities: Chlorothiazide

and Salamide by HPTLC Method; Journal of Chromatographic Science 2015;53:183–188

[9] Tengli, A.R., Shivakumar, G. and Gurupadayya, B.M. (2015) UPLCMS Method Development and Validation of Amlodipine, Hydrochlorthiazide and Losartan in Combined Tablet Dosage Form. American Journal of Analytical Chemistry, 6, 228-238. http://dx.doi.org/10.4236/ajac.2015.63021

[10] Vijaya Kumari Karra, Nageswara Rao Pilli, Jaswanth Kumar Inamadugu, and J. V. L. N. Seshagiri Rao, Simultaneous determination of losartan, losartan acid and amlodipine in human plasma by LC-MS/MS and its application to a human pharmacokinetic study, Pharm Methods. 2012 Jan-Jun; 3(1): 18–25.

[11] Wankhede SB, Raka KC, Wadkar SB, Chitlange SS, Spectrophotometric and HPLC methods for simultaneous estimation of amlodipine besilate, losartan potassium and hydrochlorothiazide in tablets, Indian journal of pharmaceutical sciences 72:1 2010 Jan pg 136-140.

[12] Shaalan RA. et al., validated stability-indicating HPLC-DAD method of analysis for the antihypertensive triple mixture of amlodipine besylate, valsartan and hydrochlorothiazide in their tablets. Arab. J. Chem. 2013. http://dx.doi.org/10.1016/j.arabjc.2013.04.012

[13] El-Shaboury SR, Hussein SA, Mohamed NA, El-Sutohy MM. Spectrofluorimetric method for determination of some angiotensin II receptor antagonists, J. Pharm. Anal. 2012;2(1):12-18.

[14] El-Gizawy SM, Abdelmageed OH, Omar MA, Deryea SM, Abdel-Megied AM. Development and validation of HPLC method for simultaneous determination of amlodipine, valsartan, hydrochlorothiazide in dosage form and spiked human plasma. Am. J. Anal. Chem.

[15] P.S. Jain, M. K. Patel, A.P. Gorle, A.J. Chaudhari and S.J. Surana, Stability-Indicating Method for Simultaneous Estimation of Olmesartan Medoxomile, Amlodipine Besylate and Hydrochlorothiazide by RP-HPLC in Tablet Dosage Form, Journal of Chromatographic Science 2012;50:680–687.

[16] Marta de Diego, Sigrid Mennickent, Juan Muñoz, Fernanda Sanhueza, Ricardo Godoy, A validated stability-indicating LC method for simultaneous determination of quinapril and hydrochlorothiazide in pharmaceutical samples, Central European Journal of Chemistry, 12(4) • 2014 • 470-475

[17] Manish Sharma, Charmy Kothari, Omkar Sherikar and Priti Mehta, Concurrent Estimation of Amlodipine Besylate, Hydrochlorothiazide and Valsartan by RP-HPLC, HPTLC and UV–Spectrophotometry, Journal of Chromatographic Science 2014;5; 2:27–35

[18] International conference on harmonization of technical requirements for registration of pharmaceuticals for human use - Q2(R1) - Parent Guideline dated 27 October 1994.