Hacettepe University Journal of the Faculty of Pharmacy
Volume 28 / Number 1 / January 2008 / pp. 15-30

Validated HPLC Method Development: The Simultaneous Analysis of Amlodipine and Valsartan in Samples for Liver Perfusion Studies

Received : 04.08.2008
Revised : 01.09.2008
Accepted : 10.09.2008

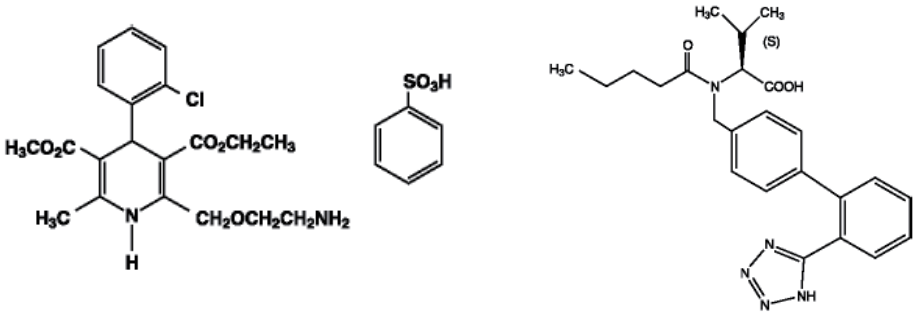
Mustafa Çelebier*, Mustafa Sinan Kaynak, Sacide Altınöz*[o], Selma Şahin****

Introduction

Amlodipine (as besylate, Fig. 1) is a long-acting calcium channel blocker used as an anti-hypertensive and in the treatment of angina. Amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart muscle [1,2]. Valsartan (Fig. 1) belongs to a class of drugs called angiotensin II receptor antagonists. Valsartan works by blocking angiotensin hormone thereby relaxing blood vessels, causing them to widen which lowers blood pressure and improves blood flow [2,3]. Valsartan is also used for the treatment of hypertension. It was noted that the combination of an angiotensin receptor blocker and a calcium channel blocker provides an effective option for patients with hypertension [4].

Several HPLC methods have been described in the literature for determination of amlodipine [5-16] and valsartan [17-29] in biological fluids. All these methods differ with respect to the mobile phase, columns and detection methods used for the analysis of compounds (Table 1 for amlodipine and Table 2 for valsartan). Although there is an LC-MS method [30] described in the literature for the simultaneous analysis of 6 beta-

* Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry 06100 Ankara / TURKEY
** Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Technology 06100 Ankara / TURKEY
[o] Corresponding Author: E-mail: saltinoz@hacettepe.edu.tr. Tel: +90 312 305 40 15 Fax: +90 312 311 47 77



Amlodipine Besylate Valsartan

Figure 1
The chemical structures of amlodipine besylate and valsartan

blockers, 3 calcium-channel antagonists (including amlodipine), 4 angiotensin-II antagonists (including valsartan) and 1 antiarrhytmic drug, this method is not specific for the determination of amlodipine and valsartan in liver perfusion studies and it requires a mass detector. Therefore, the aim of this study was to develop a new method for the simultaneous determination of these compounds by UV detection. In this study, an HPLC method was optimized and validated for this purpose. According to the ICH description, the developed method was precise and accurate [31]. After known amounts of amlodipine and valsartan were spiked into blank rat liver perfusate samples, the newly developed HPLC method was applied and it was confirmed that this method could be easily utilized for the determination of amlodipine and valsartan in real rat liver perfusate samples.

Experimental

Apparatus and Analytical Conditions

The LC system consisted of a Spectra-SYSTEM P2000 gradient pump, a SpectraSYSTEM SCM 1000 degasser, a Rheodyne manual injector with a 20 µL injection loop and a SpectraSYSTEM UV2000 detector (Thermo Separation Products, USA). The detector was set at 240 nm and peak areas were integrated automatically by an online computer equipped with ChromQuest software. The experiments were performed on a reversed-phase HICHROM Nucleosil 100-5 (Berkshire, England) C_{18} column (250

TABLE I

Reported analysis methods for amlodipine by HPLC

Samples	Column	Detection	Mobile Phase	Flow rate	Linearity	Run Time	Reference
Rat Serum	-	Amperometric	-	-	0.2-2.0 ng mL^{-1}	-	5
Human Plasma	Bond Elut C2	Amperometric	-	-	0.5-20 ng mL^{-1}	-	6
Human Plasma	10 µm Bondapak C18 (300mm x 3.9mm, i.d.)	Fluorescense	MeOH-Water (80:20 v/v)	0.8 mL min^{-1}	0.25-18 ng mL^{-1}	> 20	7
Human Plasma	C18	Mass spectrometry	MeOH - 1% HOAc (65:35 v/v)	-	-	-	8
Human Plasma	Shimpack–CLC-ODS (150 mm × 6 mm i.d. 5 µm)	Floresence (470 nm ext. ; 537 nm ems.)	sodium phosphate buffer (pH 2.5) contg. 1 mL/L triethylamine and methanol	2.8 mL min^{-1}	-	>20	9
Human Plasma	Nucleosil C8 (125 × 4.6 mm i.d.)	UV (239 nm)	0.01 M sodium dihydrogen phosphate buffer and acetonitrile (63:37 v/v) adjusted to pH 3.5	1.5 mL min^{-1}	0.5-16 ng mL^{-1}	-	10
Human Plasma	Waters symmetry C18 (5 µm, 150 × 4.6mm i.d.)	Mass spectrometry	water–acetonitrile–formic acid (30:70:0.03, v/v)	1.0 mL min^{-1}	50-10,00 pg mL^{-1}	1.5 min	11
Human Plasma	Hypersil BDS C18	Mass spectrometry	-	-	0.1-10.0 ng mL^{-1}	3.2 min	12
Human Plasma	ACQUITY UPLC BEH C18 (50 mm × 2.1 mm, i.d., 1.7 µm)	Mass spectrometry	Water and acetonitrile under gradient conditions (both contg. 0.3% formic acid)	0.35 mL min^{-1}	0.15-16.0 ng mL^{-1}	-	13
Human Plasma	C18 (250 mm x 4.6 mm, 5 µm)	Mass spectrometry	ammonium acetate (pH 3.5) 10 mmol/L-1-methanol (20:80 v/v)	1 mL min^{-1}	0.12-11.9 ng mL^{-1}	-	14
Human Plasma	C8 (4.6 mm × 150 mm, 5 µm)	UV (238 nm)	Methanol - 20 mmol L^{-1} potassium dihydrogen phosp. (pH 3.5) (42:58, v/v)	1 mL min^{-1}	0.2-20.0 ng mL^{-1}	-	16

TABLE II

Reported analysis methods for valsartan by HPLC

Samples	Column	Detection	Mobile Phase	Flow rate	Linearity	Run Time	Reference
Human Plasma	ODS Hypersil C18 5 µm	Floresence (265 nm ext. ; 378 nm ems.)	Acetonitrile - pH 2.8 phosphate buffer (50:50, v/v)	1.3 mL min^{-1}	0.2-2.0 ng mL^{-1}	-	17
Human Plasma	Phenomenex ODS (250 mm x 4.6 mm, 5 µm)	Floresence (265 nm ext. ; 378 nm ems.)	pH 2.8 phosphate buffer-MeCN (51.2:48.8 v/v)		13-2076 ng mL^{-1}	-	18
Human Urine	µBondapak C18	UV (238 nm)	acetonitrile and 0.1 M acetate buffer (pH 4.0)	Gradient elusion at different flow rates	LOQ 0.4 µg mL^{-1}	< 50 min	19
Human Plasma	µBondapak C18	Floresence (250 nm ext. ; 375 nm ems.)	Acetonitrile and 5 mM acetate buffer (pH 4.0)	(1.0-1.2 mL/min)	10-2000 ng mL^{-1}	> 15 min	20
Human Plasma	Lichrospher C-18	Floresence (265 nm ext. ; 378 nm ems.)	phosphate buffer (pH 3.1)-acetonitrile (53:47 v/v)	1.0 mL min^{-1}	5.9-2.36 ng mL^{-1}	12.5 min	21
Human Plasma	Discovery C18 (250 mm x 4.6 mm)	UV (265 nm)	phosphate buffer-acetonitrile (51.2:48.8 v/v)	1.4 mL min^{-1}	-	-	22
Human Plasma	octadecyl-silica (50 mm × 4 mm, 5 µm)	Floresence (234 nm ext. ; 375 nm ems.)	acetonitrile - 15 mM dihydrogenpotassium phosphate, pH 2.0 (45:55, v/v)	1.0 mL min^{-1}	LOQ 98 ng mL^{-1}	-	24

TABLE II

Reported analysis methods for valsartan by HPLC

Samples	Column	Detection	Mobile Phase	Flow rate	Linearity	Run Time	Reference
Human Plasma	Chromasil C18 (250 mm x 4.6 mm i.d., 5 µm)	UV (225 and 272 nm)	0.02 M sodium dihydrogen phosphate buffer (pH 3.15) and acetonitrile (58:42, v/v)	-	LOQ 500 ng mL^{-1}	-	25
Human Plasma	Atlantis dC18 (100 mm × 3.9 mm id)	Floresence (234 nm ext. ; 378 nm ems)	ACN with 0.025% TFA and a 5-mM phosphate buffer with 0.025% TFA at pH 2.5	1.3 mL min^{-1}	-	< 10 min	26
Human Plasma	-	Floresence	-	-	-	-	27
Human Plasma	C18 Atlantis (100 mm × 3.9 mm)	Floresence (234 nm ext. ; 378 nm ems.)	ACN 0.025% TFA and phosphate buffer (5 mM, pH = 2.5) 0.025% TFA	1.3 mL min^{-1}	-	< 10 min	28
Human Urine	Atlantis dC18	UV (232 nm)	mixture of acetonitrile 0.1% TFA and water 0.1% TFA	1.0 mL min^{-1}	LOQ 85 ng mL^{-1}	< 16 min	29

mm x 4.6 mm I.D.). The LC system was operated isocratically at room temperature using a mobile phase consisted of phosphate buffer (pH 3.6; 0.01 M): acetonitrile: methanol (50:40:10 v/v). After mixing, the mobile phase was filtered through a 0.45 µm membrane filter and run at a flow rate of 1.0 $mL.min^{-1}$.

Chemicals

Methanol and acetonitrile were of analytical reagent grade from Merck (Darmstadt, Germany). Milli-Q water system (Barnstead, USA) was used for the preparation of buffer and other aqueous solutions.

Standard solutions

Standard stock solution of amlodipine (as besylate) and valsartan were prepared as 100 $\mu g.mL^{-1}$ in methanol. For this purpose, 100 µg of amlodipine besylate and valsartan were dissolved in two different 100 mL of volumetric flasks. The standard stock solutions were kept in dark at 4 ^0C. Standard solutions were prepared daily by appropriate dilution of the stock solution with mobile phase.

Preparation of Perfusion Medium

The perfusion medium was Krebs bicarbonate buffer (mM; NaCl, 118; $NaHCO_3$, 24.9; $CaCl_2.6H_2O$, 2.5; KCl, 4.7; $MgSO_4.7H_2O$, 1.2; KH_2PO_4, 1.2) containing 16.7 mM glucose and 0.01 mM sodium taurocholate. Freshly prepared perfusion medium was filtered through 0.22 µm filter and then adjusted to pH 7.4 after at least 30 min oxygenation with 95% O_2 and 5% CO_2.

Phosphate buffer

1.74 g of K_2HPO_4 was dissolved in 1 L of deionized water and pH was adjusted to 3.6 by ortho-phosphoric acid.

Sample preparation

Under intraperitoneal anaesthesia, the portal vein of a male rat was cannulated with a 16GA catheter. The liver was perfused (15 $mL.min^{-1}$)

in a single-pass mode with Krebs bicarbonate buffer. After stabilization of the exposed liver (15-20 min), the blank perfusate was collected via a tubing inserted into the thoracic vena cava [32]. All spiked samples were prepared by adding 50 µL of 100 $\mu g.mL^{-1}$ standard stock solutions of amlodipine and valsartan to the 100 µL of blank rat liver perfusate and making up the volume to 1000 µL by mobile phase. The samples were filtered through a 0.45 µm membrane filter and then 20 µL injected into the LC system.

Results and Discussion

Method optimization

Various mobile phase combinations were tried initially to separate amlodipine and valsartan on C_{18} column. Preliminary experiments indicated that using different concentrations of acetonitrile or methanol with water was not able to separate the peaks of amlodipine and valsartan or to obtain suitable retention and peak shape. In order to achieve acceptable peak shapes and perform the separation on a suitable run time, various buffer systems were tried systematically. The mixture of acetonitrile and phosphate buffer (pH 3.6; 0.01 M) was capable of a good separation and short run time. The retention times of amlodipine and valsartan obtained for different phosphate buffer: acetonitrile ratios (40:60, 45:55, 50:50, 55:45, 60:40 v/v) indicated that the resolution between amlodipine and valsartan increased using higher phosphate buffer ratio (Fig. 2). However, even for a phosphate buffer: acetonitrile ratio of 60:40 (v/v), it was not possible to prevent interference between amlodipine and matrix components (rat liver perfusate). Since the retention time of valsartan increased more than desired, it was not suitable to raise the phosphate buffer proportion more than 60%. Therefore, it was decided to add another organic solvent, methanol, to the mobile phase. With methanol addition, the peaks of both amlodipine and valsartan were eluted properly and also there was no interference between amlodipine and matrix components. Thus, the final composition of the mobile phase was chosen as phosphate buffer (pH 3.6; 0.01 M), acetonitrile and methanol (50:40:10; v/v) and defined as the optimum conditions. The retention times for amlodipine and valsartan using optimum conditions were 8.23 and 10.43 minutes, respectively. For both compounds, the peak symmetries were <

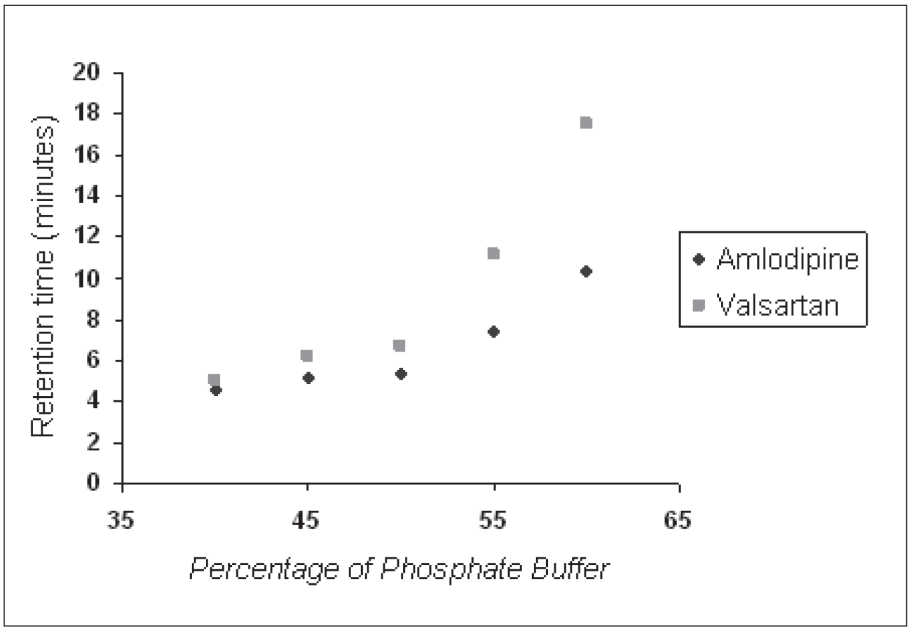


Figure 2
Effect of phosphate buffer (pH 3.6; 0.01M) proportion in the mobile phase on the separation of amlodipine and valsartan
Phosphate buffer:acetonitrile ratios (40:60, 45:55, 50:50, 55:45, 60:40 v/v)

1.5 and the theoretical plates numbers were > 2200. These values within the acceptable range of USP definition and the chromatograms taken under optimum conditions (Fig. 3) clearly show the ability of the method to assess the analyte in the presence of matrix components.

Method validation

Stability

The standard stock solutions of amlodipine and valsartan were stored at +4 °C for 1 month and protected from daylight. During this period, it was analyzed periodically and no unexpected peak appeared which might indicate the degradation. In addition, the peak areas were not changed remarkably for both compounds when they were compared with freshly prepared ones.

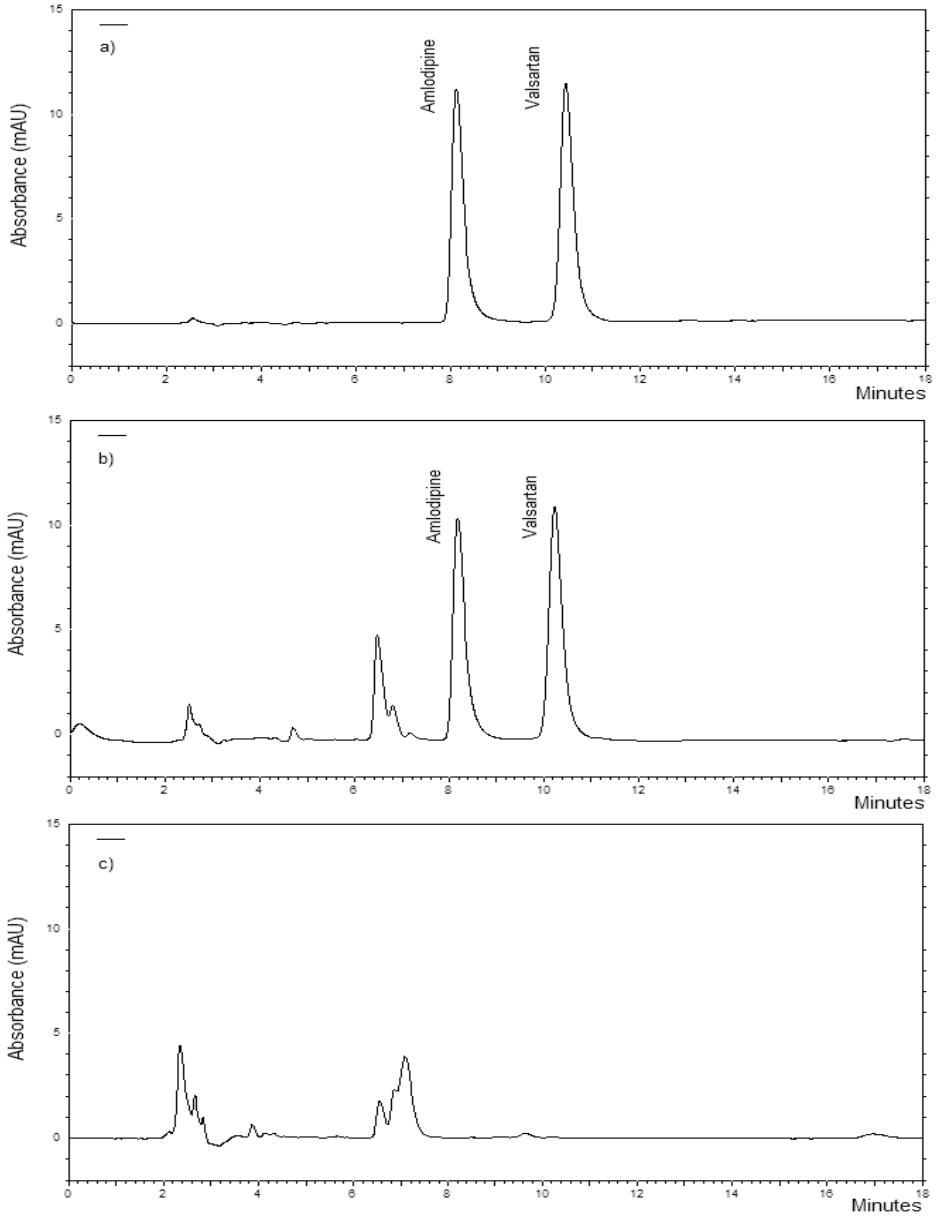


Figure 3
The chromatograms obtained in optimum conditions (a: amlodipine and valsartan standard solutions (5 µg mL^{-1}); b: spiked sample; c: blank perfusate solution)

Linearity

The calibration curves for amlodipine and valsartan were constructed under optimum conditions and the linearity of the method was determined by performing injections at eight different concentration levels in the linear range over 6 different days. The peak areas of amlodipine and valsartan were plotted against the corresponding nominal concentration to obtain calibration graph. The peak shapes and symmetries worsened after injection of 60.00 µg.mL^{-1} concentration for both compounds. Thus, the method was evaluated linear in the range of 0.05 to 60.00 µg.mL^{-1} for both amlodipine and valsartan. The regression equation data are given in Table 3.

Sensitivity

The sensitivity of the analytical method was evaluated by determining the limits of detection (LOD) and quantitation (LOQ). The signal-to-noise ratios of 3:1 and 10:1 were taken as LOD and LOQ, respectively [33, 34]. The values of LOD and LOQ for both amlodipine and valsartan were 0.02 and 0.05 µg.mL–1, respectively and given in Table 3.

Repeatability

In order to measure repeatability of the developed HPLC method, 10 consecutive injections were made with standard solutions containing

TABLE III

Linearity data of the developed method (n=6)

	Amlodipine Bes.	Valsartan
Regression equation*	y = 45477 x – 6035	y = 48114 x – 6768
Standard error of intercept	424	494
Standard error of slope	2116	3565
Correlation coefficient (r)	0,9996	0,9995
Linearity range (µg mL^{-1})	0.05 - 60	0.05 - 60
Number of data points	8	8
LOD (µg mL^{-1})	0.02	0.02
LOQ (µg mL^{-1})	0.05	0.05

*where y is peak area and x is concentration in µg mL^{-1} of amlodipine bes. and valsartan

10.00 µg mL^{-1} of amlodipine and valsartan. The results were evaluated by considering retention time and peak area. Neither the peak areas, nor the retention times changed more than % 2, which shows the high repeatability of the method.

Precision and Accuracy

Three different concentrations of standard amlodipine and valsartan solutions (within the linear range) were analyzed three consecutive days (inter-day precision) and three times within the same day (intra-day precision). The relative standard deviation (RSD) and the Bias of intra- and inter-day studies were within the acceptable range (<%5) indicating that the precision and accuracy of the method were satisfactory [33,34]. The results are also given in Table 4.

TABLE IV

Precision and accuracy of the developed method

		Intra-day			Inter-day		
	Added (µg mL^{-1})	Found* (µg mL^{-1})	Precision** RSD %	Accuracy Bias %	Found* (µg mL^{-1})	Precision** RSD %	Accuracy Bias %
Amlodipine Bes.	1	1.08 ± 0.03	4.55	8.33	1.10 ± 0.03	5.02	9.67
	10	9.79 ± 0.11	1.91	-2.10	10.24 ± 0.17	2.89	2.40
	40	39.72 ± 0.47	2.05	-0.70	41.06 ± 0.66	2.79	2.65
Valsartan	1	1.13 ± 0.03	4.19	12.67	1.12 ± 0.03	4.39	12.33
	10	9.90 ± 0.13	2.20	-1.03	10.14 ± 0.17	2.93	1.37
	40	40.08 ± 0.52	2.25	0.20	40.36 ± 1.10	4.74	0.89

* mean ± standard error (n=3)
** Relative standard deviation
Bias %: [(Found - Added) / Added] x 100

Selectivity

The chromatogram obtained from standard solution was identical with that obtained from spiked solution containing an equivalent concentration of amlodipine and valsartan. The representative chromatograms (Fig. 3) show no other peaks on the retention time of amlodipine and valsartan and the retention times did not change. Accordingly, the proposed methods can be considered selective.

Sample analysis

The spiked amlodipine and valsartan samples (5 $\mu g.mL^{-1}$; n=5) were analyzed using the developed HPLC method. The mean values determined were 4.82 ± 0.11 $\mu g.mL^{-1}$ for amlodipine and 5.08 ± 0.07 $\mu g.mL^{-1}$ for valsartan, while the RSD values were 5.25% for amlodipine and 3.35% for valsartan. The Bias values were 3.35% for amlodipine and -1.72% for valsartan.

Although there is an LC-MS method [30] described in the literature for the simultaneous analysis of 6 beta-blockers, 3 calcium-channel antagonists (including amlodipine), 4 angiotensin-II antagonists (including valsartan) and 1 antiarrhytmic drug, our method has brought simplicity for simultaneous determination of amlodipine and valsartan in biological samples. Our method does not require preanalytical extraction procedures, complex mobile phase mixtures and mass spectroscopic analysis. Moreover, the analysis can be easily carried out at ambient temperature.

Conclusion

In this study a simple, fast and reliable HPLC method was developed and validated for the simultaneous determination of amlodipine and valsartan in spiked liver perfusion samples. The developed method was successfully applied for the analysis of amlodipine and valsartan in spiked rat liver perfusion samples indicating that it could be easily applied to the *in situ* rat liver perfusion studies. The method shows a good performance with respect to linearity, sensitivity, accuracy, precision, selectivity. By this way, it provides the necessaries in order to determine the amount of amlodipine and valsartan for liver perfusion studies to investigate the pharmacokinetic profiles of these compounds.

Summary

Validated HPLC Method Development: The Simultaneous Analysis of Amlodipine and Valsartan in Samples for Liver Perfusion Studies

Amlodipine (as besylate salt) is a long-acting calcium channel blocker used as an anti-hypertensive and in the treatment of angina. Valsartan, an angiotensin II receptor antagonist, works by blocking angiotensin hormone thereby relaxing blood vessels, causing them to widen which lowers blood pressure and improves blood flow. It was noted that the combination of an angiotensin receptor blocker and a calcium channel blocker provides an effective option for patients with hypertension. The aim of this study was to develop an HPLC method for simultaneous determination of these compounds. The experiments were performed on a reversed-phase HICHROM Nucleosil 100-5 C_{18} column (250 mm x 4.6 mm I.D.). The LC system was operated isocratically at room temperature using a mobile phase consisted of phosphate buffer (pH 3.6, 0.01 M): acetonitrile: methanol (50:40:10 v/v). After mixing, the mobile phase was filtered through a 0.45 µm membrane filters and run at a flow rate of 1.0 $mL.min^{-1}$. The detector was set at 240 nm and peak areas were integrated automatically. Under these conditions, the retention times for amlodipine and valsartan were 8.23 and 10.43 minutes, respectively. The newly developed method was found to be linear within the range of 0.05 to 60.00 $\mu g.mL^{-1}$, LOD and LOQ values were 0.02 and 0.05 $\mu g.mL^{-1}$ for both amlodipine and valsartan. The standard deviation of intra- and inter-day precision studies was within the acceptable range (<5%). Our findings indicated that, the developed HPLC method was precise, accurate, specific and sensitive for the simultaneous determination of amlodipine and valsartan. In conclusion, the developed method was successfully applied for the analysis of amlodipine and valsartan in spiked rat liver perfusion samples indicating that it could be easily applied to the *in situ* rat liver perfusion studies.

Keywords: Amlodipine, Valsartan, HPLC method development, Validation, Rat liver perfusion study

Özet

Valide Edilmiş HPLC Yöntemi Geliştirilmesi: Karaciğer Perfüzyon Çalışmaları İçin Numunelerden Amlodipin ve Valsartan'ın Aynı Anda Analizi

Amlodipin (besilat tuzu şeklinde), yüksek tansiyonu önleyici ve anjina tedavisinde kullanılan uzun etkili bir kalsiyum kanal blokörüdür. Valsartan, anjiyotensin hormonunu bloke eden ve bu yolla kan damarlarını rahatlatan, onları genişleterek kan basıncını düşüren ve kan akışını iyileştiren bir anjiyotensin II reseptör antagonistidir. Bir anjiyotensin reseptör blokörü ve kalsiyum kanal blokörünün kombinasyonunun, yüksek tansiyon hastaları için etkili bir opsiyon sağladığı bildirilmiştir. Bu çalışmanın amacı, sözü edilen bileşiklerin aynı anda analizini gerçekleştiren bir HPLC yöntemi geliştirmektir. Çalışmalar, ters-faz HICHROM Nucleosil 100-5 C_{18} kolon (250 mm x 4,6 mm I.D.) kullanılarak gerçekleştirilmiştir. LC sistemi, oda sıcaklığında izokratik olarak çalıştırılmış ve fosfat tamponu (pH 3,6, 0,01 M): asetonitril:metanol (50:40:10 v/v) karışımı, hareketli faz olarak kullanılmıştır. Hareketli faz, karıştırıldıktan sonra 0,45 µm membran filtreden süzülmüş ve 1,0 $mL.min^{-1}$ akış hızında geçirilmiştir. Dedektör, 240 nm' ye ayarlanmış ve pik alanları otomatik olarak integre edilmiştir. Bu koşullar altında amlodipin ve valsartanın her ikisi için alıkonma zamanları sırasıyla 8,23 ve 10,43 dakikadır. Yeni geliştirilen metod amlodipin ve valsartan için 0,05 – 60,00 $\mu g.mL^{-1}$ aralığında lineer bulunmuş, LOD ve LOQ değerleri 0,02 ve 0,05 $\mu g.mL^{-1}$ olarak hesaplanmıştır. Gün içi ve günler arası kesinlik çalışmalarının standart sapma değerleri kabul edilebilir sınırlar içerisindedir (<%5). Sonuçlarımız, geliştirilen HPLC yönteminin amlodipin ve valsartanın aynı anda tayin edilebileceği kesin, doğru, özgün ve hassas bir yöntem olduğunu göstermektedir. Sonuç olarak, geliştirilen yöntem, amlodipin ve valsartanın spayk edildiği sıçan karaciğeri perfüzat numunelerinden analiz için başarıyla uygulanmıştır ve bu göstermektedir ki; aynı yöntem kolaylıkla *in situ* sıçan karaciğeri perfüzyon çalışmalarına uygulanabilir.

Anahtar Kelimeler: Amlodipin, Valsartan, HPLC yöntem geliştirilmesi, Validasyon, Sıçan karaciğeri perfüzyon çalışması

REFERENCES

1. Murdoch, D. and Heel, R.C.: Amlodipine. A review of its pharmacodynamic and pharmaockinetic properties, and therapeutic use in cardiovascular disease. Drugs 41, 478-505 (1991)
2. World Wide Web : www.drugs.com (Date :15.05.2008)
3. Remuzzi, A., Perico, N., Remuzzi, G.: Pharmacological and clinical profile of valsartan. Drugs of Today 34, 973 (1998)
4. Plosker, G.L. and Robinson, D.M.: Amlodipine/ valsartan : fixed-dose combination in hypertension. Drugs 68 373 (2008)
5. Shimooka, K., Sawada, Y., Tatematsu, H.: Analysis of amlodipine in serum by a sensitive high-performance liquid chromatographic method with amperometric detection. Journal of Pharmaceutical and Biomedical Analysis 7(11), 1267 (1989)
6. Josefsson, M., Zackrisson, A.L, Norlander, B.: Sensitive high-performance liquid chromatographic analysis of amlodipine in human plasma with amperometric detection and a single-step solid-phase sample preparation. Journal of Chromatography, B: Biomedical Applications 672(2), 310 (1995)
7. Tatar, S., Atmaca, S.: Determination of amlodipine in human plasma by high-performance liquid chromatography with fluorescence detection. Journal of Chromatography, B: Biomedical Sciences and Applications 758(2), 305 (2001)
8. Feng, Y., Zhang, L., Shen, Z., Pan, F., Zhang, Z.: Analysis of amlodipine in human plasma by liquid chromatography-mass spectrometry. Journal of Chromatographic Science 40(1), 49 (2002)

90 Bahrami, Gh; Mirzaeei, Sh: Simple and rapid HPLC method for determination of amlodipine in human serum with fluorescence detection and its use in pharmacokinetic studies. Journal of Pharmaceutical and Biomedical Analysis 36(1), 163 (2004)

10. Zarghi, A., Foroutan, S.M., Shafaati, A., Khoddam, A.: Validated HPLC method for determination of amlodipine in human plasma and its application to pharmacokinetic studies. Farmaco 60(9), 789 (2005)
11. Nirogi, R.V.S., Kandikere, V.N., Mudigonda, K., Shukla, M., Maurya, S.: Sensitive and rapid liquid chromatography/tandem mass spectrometry assay for the quantification of amlodipine in human plasma. Biomedical Chromatography 20(9), 833 (2006)
12. Bhatt, J., Singh, S., Subbaiah, G., Shah, B., Kambli, S., Ameta, S.: A rapid and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the estimation of Amlodipine in human plasma. Biomedical Chromatography 21(2), 169 (2007)
13. Ma, Y., Qin, F., Sun, X., Lu, X., Li.: Determination and pharmacokinetic study of amlodipine in human plasma by ultra performance liquid chromatography-electrospray ionization mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 43(4), 1540 (2007)
14. Du, G., Liu, Y., Liu, D.: Determination of the amlodipine in plasma by LC-MS. Zhongguo Yaoshi (Wuhan, China) 10(4), 328 (2007)
15. Zou, J., Zhu, Y., Yu, C., Fan, H., Xiao, D., Sheng, H., Wang, W.: Determination of amlodipine in human plasma by HPLC-MS and study on its bioequivalence. Zhongguo Linchuang Yaoxue Zazhi 16(4), 214 (2007)
16. Chen, L., Wu, W.: Determination of concentration of (S)-amlodipine benzenesulfonate in human blood plasma with reversed-phase high-performance liquid chromatography (RP-HPLC). Yiyao Daobao 25(12), 1256 (2006)
17. Sioufi, A., Marfil, F., Godbillon, J.: Automated determination of an angiotensin II receptor antagonist, CGP 48 933, in plasma by high-performance liquid chromatography. Journal of Liquid Chromatography 17(10), 2179 (1994)
18. Li, Y., Zhao, Z., Chen, X., Wang, J., Guo, J., Xiao, F.: HPLC determination of valsartan

in human plasma. Yaowu Fenxi Zazhi 20(6), 404 (2000)

19. Gonzalez, L., Alonso, R.M., Jimenez, R.M.: A high-performance liquid chromatographic method for screening angiotensin II receptor antagonists in human urine. Chromatographia 52(11/12), 735 (2000)
20. Gonzalez, L., Lopez, J.A., Alonso, R.M., Jimenez, R.M.: Fast screening method for the determination of angiotensin II receptor antagonists in human plasma by high-performance liquid chromatography with fluorimetric detection. Journal of Chromatography, A 949(1-2), 49 (2002)
21. Xu, Y., Cao, W., Lin, X., Ling , S.: Determination of valsartan in human plasma by HPLC. Zhongguo Yaolixue Tongbao 18(5), 586 (2002)
22. Zhang, J., Chen, S., Gu, T., Lu, X., Hu, Y.: Pharmacokinetics and pharmacodynamics of valsartan in hypertensive patients with normal and impaired renal functions. Shanghai Dier Yike Daxue Xuebao 24(2), 133, 142 (2004)
23. Li, Y., Chen, J., Wang, De., Li, K., Liu, L., Sun, C.: Quantification of plasma valsartan and hydrochlorothiazide by HPLC. Zhongguo Xinyao Zazhi 14(11), 1335 (2005)
24. Macek, J., Klima, J., Ptacek, P.: Rapid determination of valsartan in human plasma by protein precipitation and high-performance liquid chromatography. Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences 832(1), 169 (2006)
25. Kocyigit-Kaymakcıoglu, B., Unsalan, S., Rollas, S.: Determination and validation of ketoprofen, pantoprazole and valsartan together in human plasma by high performance liquid chromatography. Pharmazie 61(7), 586 (2006)
26. Iriarte, G., Ferreiros, N., Ibarrondo, I., Alonso, R.M., Maguregi, M.I., Gonzalez, L., Jimenez, R.M.: Optimization via experimental design of an SPE-HPLC-UV-fluorescence method for the determination of valsartan and its metabolite in human plasma samples. Journal of Separation Science 29(15), 2265 (2006)
27. Li, Y., Yang, L.: Determination of blood concentration of valsartan by HPLC fluorescence method. Yixue Linchuang Yanjiu 23(10), 1684 (2006)
28. Iriarte, G., Ferreiros, N., Ibarrondo, I., Alonso, R.M., Maguregui, M. I., Jimenez, R.M.: Biovalidation of an SPE-HPLC-UV-fluorescence method for the determination of valsartan and its metabolite valeryl-4-hydroxy-valsartan in human plasma. Journal of Separation Science 30(14), 2231 (2007)
29. Ferreiros, N., Iriarte, G., Alonso, R.M., Jimenez, R.M., Ortiz, E.: Separation and quantitation of several angiotensin II receptor antagonist drugs in human urine by a SPE-HPLC-DAD method. Journal of Separation Science 31(4), 667 (2008)
30. Lena, K., Elisabeth, L.Ø., Mimi, S.O., Mette K., Elsa, L., Asbjørg, S.C.: Simultaneous determination of 6 beta-blockers, 3 calcium-channel antagonists, 4 angiotensin-II antagonists and 1 antiarrhytmic drug in post-mortem whole blood by automated solid phase extraction and liquid chromatography mass spectrometry. Method development and robustness testing by experimental design. Journal of Chromatography B, 850, 147 (2007)
31. ICH Steering Committee. Validation of analytical procedures: Text and Methodology Q2(R1). Harmonized Tripartite Guideline. 2005
32. Sahin, S. and Rowland, M.: Development of an optimal method for the dual perfusion of the isolated rat liver. Journal of Pharmacological and Toxicological Methods 39, 35 (1996)
33. Taverniers, I. and Loose, M.D., Bockstaele, E.V.: Trends in quality in the analytical laboratory. I. Traceability and measurement uncertainty of analytical results. Trends in Analytical Chemistry 23, 480 (2004)
34. Taverniers, I. and Loose, M.D., Bockstaele, E.V.: Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. Trends in Analytical Chemistry 23, 535 (2004)