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Research Article

Simultaneous Determination of Some Selected Hypertension Drugs in Hospital Effluents by Using LCMS

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Abstract

A robust method was developed and validated for the determination of Bisoprolol, Losartan, Amlodipine, Atorvastatin and Candesartan antihypertension drugs in hospital wastewater. The method employs acetonitrile/formic acid as a mobile phase in a gradient mode, flow rate 1ml/min. The column used was ODS Shimpack 4.6*150*5µm and the injected volume was 5µL. Solid phase extraction was used for sample cleaning before analysis. Analysis time was less than seven minutes. The limits of quantitation (LOQ) ranges were 0.001-0.003 and 0.003-0.014 ug/ml, respectively. Acceptable recoveries were achieved for the drug standards. This new method proved to be simple, robust, precise and accurate.

Keywords: LCMS; Method development; Hypertension drugs

Introduction

Pharmaceuticals are emerging as potential pollutants of the environment and have become a considerable source of environmental contamination [1–4]. A number of therapeutic pharmaceuticals are used in large quantities and may be present in effluents at varying concentrations. The health impact of long-term exposure to mixtures of these compounds is unknown. Sources of pharmaceutical pollution of the environment include humans, hospitals, livestock and pharmaceutical industry. They can enter the waterways through their disposal and as a result can become a serious and persisting health hazard to human health, as well as to the life of the flora and fauna. The presence of pharmaceuticals in the environment raised great concern in the recent years regarding their potential impact as they can affect water quality by the increasing discharge of drugs. This issue has prompted numerous monitoring and assessment programs and studies [5–9].

The determination of pharmaceuticals at trace levels in various environmental matrices, especially water, is mainly attributable to the advancement of the sensitivity and accuracy of the new generations of analytical instrumentation. For instance, high performance liquid chromatography (HPLC) coupled to mass spectrometry, is a common technique of choice for various drug residues and pharmaceuticals [2,10–15]. Anti-Hypertensive drugs are pharmaceutical used to help control blood pressure. Untreated high blood pressure can lead to diseases of the heart, arteries, kidney damage, or stroke [16–18]. In this study, we report the development and validation of an HPLC-MS method enabling to quantify simultaneously some selected antihypertensive drugs i.e. Bisoprolol, Losartan, Amlodipine, Atorvastatin and Candesartan.

Materials and Methods

Drugs standard Bisoprolol, Losartan, Amlodipine, Atorvastatin and Candesartan were kindly donated by Kingstone Pharmaceuticals, Sudan, Acetonitrile, (Carlo Erba, France), formic acid, acetone 99% triethylamine methanol (99%) sulphoric acid (98%), ethanol (99%) (SDFCL, India). Deionized water, filter paper (Watman 100 and 0.45 micron filter paper. All chemical were analytical or HPLC grade.

Preparation of standard stock solution

Exactly 0.01g from each standard was weighed separately in a beaker (50mL) and 10ml of the diluent was added. The standard was transferred to ultrasonic bath for 5min and then was completed to the mark to obtain a concentration of 200mg/ml. From this standard solution, 0.5ml were pipetted into 10ml volumetric flask and then completed to the mark by the diluent to obtain a final solution of a concentration of 10ug/ml. Further dilutions were made from this solutions for the different validation tests.

Samples pretreatment and clean up

Initially, 500 ml of each sample of wastewater was filtered through Watman filter paper (100) and then through 0.45 micron, then acidified to pH 3.0 by adding sulphuric acid. The sample was passed through C-18 cartridge activated with 5ml of methanol, 5ml methanol/water 50/50 (v/v) and 5ml of water at (pH 3). The cartridge was washed further with 5ml of acidified water (pH 3).The cartridge was eluted with 5ml of triethylamine (5% v/v) in methanol. The eluted solution was evaporated at normal room temperature (20^0C). Finally, sample volume was completed to 1ml by adding water/acetonitrile 95/5 (v/v).

Mobile phase preparation of mobile phase

Gradient elution mode was used in the analysis. Mobile phase A (1% formic acid) and mobile phase B was acetonitrile. The gradient program is shown in Table 1.

Table 1: The Gradient elution program.

Time (min)	A	B
0.01	70	30
3	40	60
5	20	80
8	70	30
9	70	30
10	stop	

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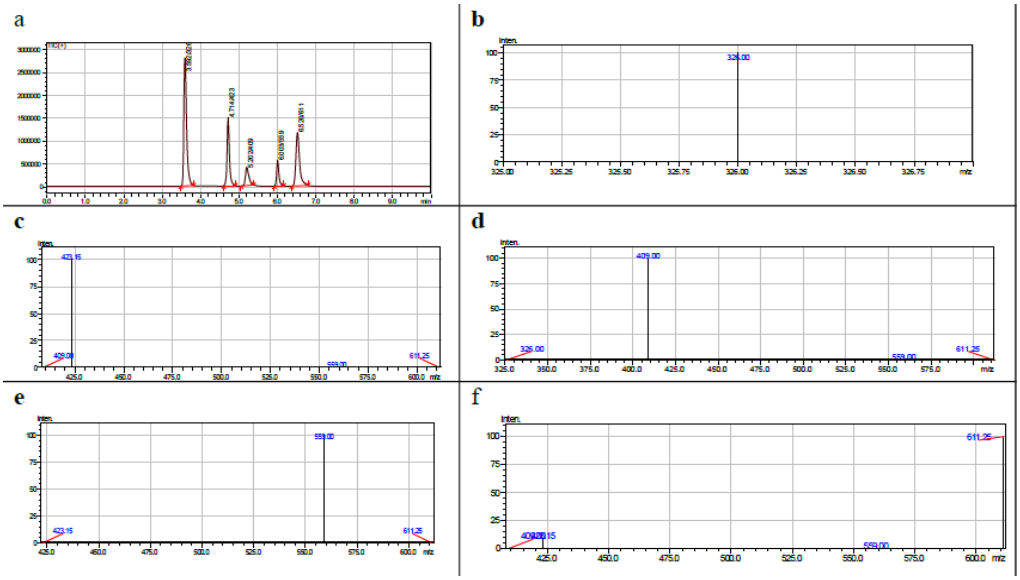

Figure 1: a) The chromatogram of the drugs; b) SIM spectra of Bisoprolol; c) Losartan; d) Amlodipine; e) Atorvastatin; f) Candesartan.

Preparation of the diluents: The diluent was made from formic acid 1% and acetonitrile (50%, 50%).

HPLC conditions: HPLC column was: ODS Shimpack 4.6*150*5µm,-flow rate : 1mL/min, injection volume: 5µL

MS condition: Drying gas: N2 15L/min, nebulizing gas N2 1.5L/min, interface temperature: 350^{0}C heat block: 200^{0}C, the scan mode: +ve SIM, and the drugs selected ions (M+H) used in the analysis were as follows: Bisoprolol (326), Losartan (423.15), Amlodipine (409), Atorvastatin (559) and Candesartan (611).

Results and Discussion

LC-MS optimization

Several gradient programs were tried to achieve optimum separation of the entire drug standards. Gradient elution was necessary to avoid excessive retention. Well-resolved peaks were obtained within short analysis time. The positive and the negative electrospray ionization (ESI) modes were investigated for attaining the highest sensitivity during the method development. The full scan of the drugs mixture in positive mode showed that the signal-to- noise ratios obtained in this mode were higher than those of the in negative mode. Hence, positive mode was used to obtain the precursor ion [M+H] for the analysis. During the method development, the quadrupole mass analyzers operated in selected ion monitoring (SIM) mode where it monitors only a few mass-to- charge ratios. By setting the optimal chromatographic parameters and using electrospray ionization, the produced chromatogram and SIM spectra of the drug standards are shown in Figure 1. The retention times of Bisoprolol, Losartan, Amlodipine, Atorvastatin and Candesartan were 3.59, 4.71, 5.17, 6.00 and 6.49 min, respectively.

Method validation

Test method validation is a set of Comprehensive experiments that evaluate and document the performance of an assay. These experiments are designed to demonstrate the scientific validity of

Table 2: System Suitability test results.

	Theoretical plates	Tailing factor	Retention time	Resolution
Bisoprolol	10826	1.57	3.59	--
Losartan	22718	1.244	4.71	8.504
Amlodipine	12353	1.485	5.17	3.114
Atorvastatin	37869	1.479	6	5.731
Candesartan	17739	1.617	6.49	3.245

Table 3: linearity regression equations of the drugs.

Drug	Regression equation	R2
Bisoprolol	y = 7E+06x + 1833.	R^2 = 0.998
Losartan	**y = 3E+06x - 30225**	R^2 = 0.998
Amlodipine	y = 1E+06x - 26297	R^2 = 0.998
Atorvastatin	y = 1E+06x - 22014	R^2 = 0.998
Candesartan	y = 4E+06x + 63628	R^2 = 0.998

Table 4: Accuracy test results.

Drug	AVG RE 80%	AVG RE 100%	AVG RE 120 %
Bisoprolol	98.17	97.87	98.73
Losartan	98.8	98.31	98.85
Amlodipine	97.58	97.67	98.87
Atorvastatin	99.06	100.28	99.5
Candesartan	99.18	99.62	99.01

results produced by the method during routine sample analysis. Establishing that a test method consistently produces reliable analytical results is a critical element of assuring the quality and safety of pharmaceutical products. In this work validation was performed according to the ICH guidelines [19].

System suitability testing: As an essential part of the method development, system suitability was studied to interpret the

Table 5: Intraday Precision test results.

	Drug	Bisoprolol	RSD	Amlodipine	Atorvastatin	Candesartan
	Conc (ug/ml)	RSD	Losartan	RSD	RSD	RSD
Day1	0.4	1.1	0.5	0.76	1.03	0.67
	1		1.6	1.25	0.44	0.73
		1.5	1			
	2		1.1	0.76	1.25	0.49
		0.83	9			
Day2	0.4		0.7	0.77	1.37	1.3
		1.22	1			
	1		1.3	1.4	0.66	1.14
		0.58	8			
	2		1.1	1.05	1.79	1.35
		0.38	9			
Day3	0.4		1.5	1.15	1.37	0.52
		0.94	9			
	1		1.6	1.24	1.32	0.97
		1.06	5			
	2		0.8	0.66	1.3	0.92
		0.68	2			

Table 6: Interday precision test results.

Drug Conc (ug/ml)	Bisoprolol	RSD	Amlodipine	Atorvastatin	Candesartan
	RSD	Losartan	RSD	RSD	RSD
0.4		0.9	0.89	1.26	0.83
	1.09	3			
1	0.8	1.4	1.13	1.08	0.87
	2	1			
2	0.6	1	0.82	1.45	0.92
	3	7			

chromatographic performance of the instrument. Resolution, tailing factor, number of theoretical plates, peak width and height equivalent to a theoretical plate (Table 2).

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which might be expected to be present. The chromatogram and peaks of interest were investigated for the presence of any interferent peaks. No such observations were noticed in the obtained chromatogram.

Linearity: Linearity is the ability of the method to return values that are directly proportional to the concentration of the drugs in the sample. The linearity plot was examined over a number of concentration levels (0.1, 0.4, 1, 1.5 and 2ug/ml) of the standard drug solutions. Each solution was injected in triplicate and average values were used as representative. Slope, intercept and correlation coefficients, the slope and the intercept, were calculated using Lab solution software. The data obtained and the regression lines obtained for each drug are shown in Table 3.

Accuracy and recovery: Recovery studies were performed to determine accuracy. A standard solution of Bisoprolol was were spiked with known amounts at 3 different levels (80%, 100% and 120%). The solutions were analyzed and the mean recovery was calculated. The process was repeated for the other drugs and the results obtained are shown in Table 4.

Table 7: Limits of Detection and Quantification test results.

Drug	Selected Ion[M+H]	LOD	LOQ
Bisoprolol		0.001	0.0103
	326	0	
Losartan		0.001	0.0142
	423.15	4	
Amlodipine		0.001	0.0099
	409	0	
Atorvastatin		0	0.0031
	559	3	
Candesartan		0.003	0.0319
	611	2	

Table 8: Solution stability test results.

Drug	Theortical plates	Tailing factor	Retention time	Resolution	RSD
Bisoprolol					0.19
	9675	1.437	3.588	--	41
Losartan			4.71		0.46
	21342	1.275		8.504	59
Amlodipine					0.41
	11070	1.523	5.17	3.114	28
Atorvastatin					0.02
	33399	1.385	5.999	5.731	75
Candesartan					0.31
	15897	1.47	6.49	3.245	77

Intraday and interday precision: The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision experiments included intraday and interday studies. Results were statistically evaluated including mean, standard error of mean, standard deviation, RSD%. The results obtained are shown in Table 5 and 6.

Limits of detection and quantification: The calculated values were regarded as the lowest concentration for detection and quantitation (Table 7).

Robustness: Robustness was investigated by minor variation of the experimental conditions i.e. mobile phase composition, pH, and flow rate and column temperature intentionally. The experimental results did not show any significant difference from the initial parameters.

Solution stability: The solution stability test was performed by measuring the same samples after six hours. The results obtained in shown in Table 8.

Analysis of samples: Using this method, water samples from a effluents and drinking water sample from two hospitals in Khartoum, Sudan were collected and treated as mentioned previously and finally analysed.

The results obtained are depicted in Table 9.

Conclusion

In this paper; a robust, accurate and precise method for the determination and quantification of Bisoprolol, Losartan Amlodipine, Atorvastatin, and Candesartan was developed and validated. The most important feature of the proposed methods was its versatility. The developed method proved to be simple, accurate robust and precise.

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Table 9: Water sample analysis results.

First Sample	m/z	RT	Concentration (ug/ml)
Bisoprolol	326	3.508	0.004
Losartan	423.15	4.725	0.009
amlodipine	409	4.872	0
Atorvastatin	559	6.025	0
candesartan	611.25	6.5	0.005
Second Sample			
Bisoprolol	326	3.508	0.002
Losartan	423.15	4.725	0
Amlodipine	409	4.872	0
Atorvastatin	559	6.025	0
Candesartan	611.25	6.5	0.006
Drinking water sample			
Bisoprolol	326	3.508	0
Losartan	423.15	4.725	0
Amlodipine	409	4.872	0
Atorvastatin	559	6.025	0
Candesartan	611.25	6.5	0.006

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