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HPLC Analysis of Lisinopril in Tablets

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Abstract

HPLC method was applied for the identification of Lisinopril in trade pharmaceutical products – tablets. The HPLC analytical parameters: retention time (t_R), capacity factor for the analyte peak (k′), number of theoretical plates (N) and tailing factor for the analyte peak (T), for tablets with different content of Lisinopril: 5 mg, 10 mg, 20 mg were compared. The data for the quantity of drug and for parameters of validation procedure: repeatability (precision) and accuracy for Lisinopril tablets 10 mg, 20 mg and for model mixtures with Lisinopril, were obtained by HPLC method. The results from the analysis of Lisinopril in tablets correspond to the USP XXIV requirements.

Key words: Accuracy, HPLC analysis, Lisinopril, tablets, repeatability.

Introduction

Lisinopril blocks the active centre of ACE and by this way inhibites the conversion of angiotensin I to angiotensin II and the inactivation of bradykinin (Delgado and Remers, 1986; Fischer and Gere, 2001). For the determination of substance Lisinopril in USP XXIV Pharmacopoeia is applied HPLC method (USP XXIV, 2000) and in British Pharmacopoeia is used potentiometry (British Pharmacopoeia, 2000). In USP XXIV Pharmacopoeia is described HPLC method for the quantitative analysis of Lisinopril in tablets (USP XXIV, 2000). There are different methods developed for the assay of Lisinopril in single and multicomponent pharmaceutical dosage forms (Atmaca *et al.*, 1994; El – Yazbi *et al.*, 1999; Ozer and Senel, 1999). The most important of them are: spectrometry: for Lisinopril and Hydrochloro – thiazide in combined dosage forms (El – Yazbi *et al.*, 1999; Panzade and Mahad, 1999; El – Gindy *et al.*, 2001, 923 – 931) and for simultaneous determination of Lisinopril and Amlodipine besilate in combined tablet preparations (Jain and Agrawal, 2000; Mashru and Parikh, 2000; Prasad *et al.*, 1999); spectrometry, after derivative reactions with different reagents (Atmaca *et al.*, 1994; El – Yazbi *et al.*, 1999; El – Gindy *et al.*, 2001, 913 – 922); fluorimetry: for Lisinopril in tablets (El – Gindy *et al.*, 2001, 913 – 922; Iskender and Yarenci, 1996) and for combination of Lisinopril and Hydrochlorothiazide in tablets (El – Yazbi *et al.*, 1999); HPTLC densitometry: for Lisinopril and Hydrochlorothiazide in binary mixtures (El – Gindy *et al.*, 2001, 923 – 931); HPLC: for Lisinopril in tablets (El – Gindy *et al.*, 2001, 913 – 922).

Recently many firms produced many new pharmaceutical products – tablets with active substance Lisinopril, but with difference in content of supplements. Some of them are: Novatec, Prinil, Prinivil, Vivatec (Merck Sharp – Dohme); Acerbon, Zestril (Zeneca). Different

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supplements and the presence of impurities (2 – amino – 4 – phenylbutanoic acid; toluene – 4 sulphonic acid; cyclohexyl analogue) cause some difficulties in analysis. To control the quality of these drugs it is necessary the development of validation procedure for analytical methods.

The aim of this study is to validate the HPLC method for quantity analysis of Lisinopril in tablets, by the obtained data for parameters of validation procedure: repeatability (precision) and accuracy, in order to confirm, that this method can be applied for quality control of different new trade drug products – tablets with active substance Lisinopril, but with content of different supplements.

Materials and Methods

Drugs: Trade drug products (Adipharm) – Lisinopril tablets: 5 mg, 10 mg, 20 mg – zero series, work sample; reference standard – USP Lisinopril RS (Lisinopril Dihydrate).

Reagents: Monobasic potassium phosphate; phosphoric acid – Merck; sodium 1 – hexanesulfonate; acetonitrile for chromatography – LiChrosolv ® – Merck (Germany); bidistilled water; methanol for chromatography – LiChrosolv ® – Merck (Germany).

For the identification and determination of Lisinopril in different drug products – tablets is used modified USP Pharmakopoeial HPLC method (USP XXIV, 2000). The conditions are:

Standard preparation: An accurately weighted quantity of reference standard – USP Lisinopril RS (Lisinopril Dihydrate), was transfered to a suitable size volumetric flask and was dissolved in diluent (bidistilled water : methanol = 4 : 1) to obtain a solution, having a known concentration of 0.2 mg/ml.

Test preparation: An accurately weighted quantity of tablets, containing Lisinopril was dissolved in diluent (bidistilled water : methanol = 4 : 1) in a suitable size volumetric flask. After 5 min of sonication, the flask was shaked by mechanical means for 20 min. The test preparation was diluted with diluent to volume, to obtain a known concentration of 0.2 mg/ml, and was filtered.

Mobile phase: 1.0 g of sodium 1 – hexanesulfonate was dissolved in 800 ml of Phosphate solution. To the obtained solution was added 200 ml of acetonitrile. The mobile phase was mixed and filtered. Phosphate solution: 4.1 g of monobasic potassium phosphate was dissolved in 900 ml of bidistilled water in a 1000 ml volumetric flask. This solution was adjusted with phosphoric acid to pH = 2.0. The Phosphate solution was diluted with bidistilled water to volume and was mixed. Before using, the mobile phase was filtered through membrane filter with a pore size 0.45 μm.

Equipment: HPLC system LC – 10 Advp Shimadzu (Japan), equipped with: analytical column Spherisorb ® RP – 18, ODS 2, 4.6 mm / 250.0 mm, particle diameter: dp = 5 μm; column oven CTO – 10 Asvp Shimadzu; LC – 10 A isocratic pump; manual injector with 20 μl loop; detector SPD – 10 Avvp (UV – VIS, with fixed wavelength at 215 nm). The analysis was controlled and the data were acquired with CLASS LC – 10 A software.

Chromatographic parameters: Mobile phase: 0.125 % solution of sodium 1 – hexanesulfonate in Phosphate solution (pH = 2) : acetonitrile = 800 : 200; flow: 1.5 ml/min; column temperature: 40 °C; injection volume: 20 μl; detection: 215 nm.

Chromatographic procedure: Equal volumes of 20 µl of the Standard preparation and of the Test preparation were injected separately into the chromatograph, chromatograms were recorded and the responses for the major peaks were measured. After applying the USP HPLC assay procedure, the content of Lisinopril (mg) in the examined tablets and in model mixtures was calculated by the formula: $(L / D).C .(r_u / r_s) = (L / D).[(M_1.C_1) / M_2] . (r_u / r_s)$.

L – the labeled quantity of Lisinopril in each tablet – [mg]; D – the concentration of Lisinopril in the Test preparation, based on the labeled quantity per tablet and the extent of dilution – [0.2 mg/ml]; C – the concentration of USP Lisinopril RS in the Standard preparation (Lisinopril Dihydrate), calculated on the anhydrous basis [0.1837 mg/ml]; C_1 – the concentration of USP Lisinopril RS in the Standard preparation (Lisinopril Dihydrate), based of the extent of dilution – [0.2 mg/ml]; M_1 – molecular weight of Lisinopril – 405.4962; M_2 – molecular weight of Lisinopril Dihydrate – 441.5269; r_u – the Lisinopril peak response, obtained from the Test preparation; r_s – the Lisinopril peak response, obtained from the Standard preparation.

Results

In respect of supplements placebo solution was prepared. The specificy of the applied HPLC method is proved by the fact, that on the chromatogram, obtained with placebo solution peak did not exist.

The identity of Lisinopril in the examined tablets: 5 mg, 10 mg, 20 mg, is proved by the relevance between the retention times for tablets (t_R): 7.375 (L_t5), 7.383 (L_t10), 7.375 (L_t20) and for reference standard – USP Lisinopril RS: 7.383 (L S). For tablets and for reference standard the obtained results for some analytical parameters are: capacity factor for the analyte peak (k'): 5.9520 (L S, L_t10), 5.9440 (L_t5, L_t20); column efficiency (N): 2146 (L_t5), 2238 (L_t10), 2178 (L_t20), 2169 (L S); tailing factor for the analyte peak (T): 1.2895 (L_t5), 1.2500 (L_t10), 1.2209 (L_t20), 1.3125 (L S).

In table 1 are presented: peak's area – for Lisinopril tablets 10 mg ($r_u L_t10$), 20 mg ($r_u L_t20$) and for model mixtures with Lisinopril: I (r_u L5), II (r_u L10), III (r_u L15); Shovene's criterion for the obtained quantity in every sample – for Lisinopril tablets 10 mg ($U_{Sh} L_t10$), 20 mg ($U_{Sh} L_t20$) and for model mixtures with Lisinopril: I (U_{Sh} L5), II (U_{Sh} L10), III (U_{Sh} L15).

Table 1 Areas of peaks and Shovene 's criterion for Lisinopril tablets 10 mg ($r_u L_t 10$; $U_{Sh} L_t 10$), 20 mg ($r_u L_t 20$; $U_{Sh} L_t 20$).

Lisinopril tablets				
N : sample	r_u L_t 10	r_u L_t 20	U_{Sh} L_t 10	U_{Sh} L_t 20
1.	6 202 711	6 589 871	1.2456	0.4821
2.	6 557 725	6 557 725	0.2807	0.3036
3.	7 007 765	6 718 454	0.9474	1.1964
4.	7 072 056	6 557 725	1.1228	0.3036
5.	6 300 559	6 396 996	0.9825	0.5893
6.	6 814 891	6 204 122	0.4211	1.6607

Model mixtures with Lisinopril			
N : sample	1.	2.	3.
I r_u L 5	5 975 606	5 940 740	5 999 273
II r_u L 10	5 952 314	5 852 337	5 828 629
III r_u L 15	5 893427	5 913 040	5 913 035
I U_{Sh} L 5	0.40	1.20	0.60
II U_{Sh} L 10	1.10	0.20	0.90
III U_{Sh} L 15	1.00	1.00	0.50

Table 2. Data for the content of Lisinopril and degree of recovery for Lisinopril tablets 10 mg (L_t10; R_t10), 20 mg (L_t20; R_t20).

N:	L_t 10 [mg/tabl.]	R_t 10 [%]	L_t 20 [mg/tabl.]	R_t 20 [%]
1.	9.65	96.50	20.50	102.50
2.	10.20	102.00	20.40	102.00
3.	10.90	109.00	20.90	104.50
4.	11.00	110.00	20.40	102.00
5.	9.80	98.00	19.90	99.50
6.	10.60	106.00	19.30	96.50
$\overline{X}$ ± SD	10.36 ± 0.57		20.23 ± 0.56	
$\overline{X}$ ± RSD [%]		103.58 ± 5.46		101.17 ± 2.76
SD	0.57	5.66	0.56	2.79
RSD [%]	5.50	5.46	2.77	2.76
S $\overline{X}$	0.23	2.31	0.23	1.14
P [%]	98.00	98.00	99.90	99.90
t	3.37	3.37	6.86	6.86
t . S $\overline{X}$	0.78	7.78	1.58	7.82
$\overline{X}$ ± t . S $\overline{X}$	9.58 ÷ 11.14	95.80 ÷ 111.36	18.65 ÷ 21.81	93.35 ÷ 108.99
E [%]	2.22	2.23	1.14	1.13

Table 3. Data for the content of Lisinopril and degree of recovery for model mixtures : I (L′5; R 5), II (L′10; R 10), III (L′15; R 15).

N:	L′ 5 [mg]	R 5 [%]	L′ 10 [mg]	R 10 [%]	L′ 15 [mg]	R 15 [%]
1.	5.09	101.19	10.10	100.80	15.06	99.80
2.	5.01	100.60	9.97	99.11	15.02	100.13
3.	5.10	101.59	9.90	98.70	15.03	100.13
$\overline{X}$ ± SD	5.07 ± 0.05		9.99 ± 0.10		15.04 ± 0.02	
$\overline{X}$ ± RSD [%]		101.13 ± 0.49		99.54 ± 1.12		100.02 ± 0.19
SD	0.05	0.50	0.10	1.11	0.02	0.19
RSD [%]	0.99	0.49	1.00	1.12	0.13	0.19
S $\overline{X}$	0.03	0.29	0.06	0.64	0.01	0.11
P [%]	95.00	95.00	95.00	95.00	95.00	95.00
t	4.30	4.30	4.30	4.30	4.30	4.30
t . S $\overline{X}$	0.13	1.25	0.26	2.75	0.04	0.47
$\overline{X}$ ± t . S X	4.94 ÷ 5.20	99.88 ÷ 102.38	9.73 ÷ 10.25	96.79 ÷ 102.29	15.00 ÷ 15.08	99.55 ÷ 100.49
E [%]	0.59	0.29	0.60	0.64	0.07	0.11

In table 2 are pointed the results for the content of Lisinopril in tablets 10 mg, 20 mg and the data for the degree of recovery. The quantity of Lisinopril was calculated by using the results of areas of peaks from table 1. For every kind of the examined tablets were prepared 6 samples. In table 2 are indicated for all samples: N – number of the individual measurements (1 ÷ 6); L_t10, L_t20 – content of Lisinopril in tablets – (mg/tabl.), with labeled quantity respectively 10 mg, 20 mg; R_t10, R_t20 – degree of recovery (%); P – confidential possibility (%), t – coefficient. All of the experimental data suit confidential intervals. The results for the obtained content of Lisinopril (L′5, L′10, L′15) in the investigated model mixtures I, II, III (with content of USP Lisinopril RS – Lisinopril Dihydrate – respectively: 5 mg (50 %) – L5; 10 mg (100 %) – L10; 15 mg (150 %) – L15) and the data about the degree of recovery (R5, R10, R15) are summerized in table 3. The quantity of Lisinopril was calculated by using the results of areas of peaks from table 1. For every kind of the examined model mixtures were prepared 3 samples: L5 – 5.03, 4.98, 5.02; L10 – 10.02, 10.06, 10.03; L 15 – 15.09, 15.00, 15.01. In table 3 for all samples are indicated: N – number of the individual measurements (1 ÷ 3); L′5, L′10, L′15 – content of Lisinopril in model mixtures (mg), obtained by HPLC; R5, R10, R15 – degree of recovery (%); P – confidential possibility (%); t – coefficient.

Discussion

The USP XXIV requirements are: capacity factor for the analyte peak – ($k' \geq 2$); number of theoretical plates – ($N \geq 850$); tailing factor for the analyte peak – ($T \leq 2$)[3]. The compared results show, that for all of the examined tablets the capacity factor for the analyte peak is more than 2 – ($k' \geq 2$); the number of theoretical plates are more than 850 – ($N \geq 850$); the tailing factor for the analyte peak is less than 2 – ($T \leq 2$). These parameters for all tablets correspond to the USP XXIV requirements (USP XXIV, 2000).

The peak's area of the Standard preparation is $r_s = 5\ 905\ 167$. From table 1 it is obvious, that the data of Shovene's criterion (U_{Sh} L_t10, U_{Sh} L_t20) for tablets are lower than standard Shovene's criterion (U_{Sh} S = 1.73; N = 6) – experimental results suit standard criterion. The pointed data from table 2 show, that the results of quantity analysis of drug in Lisinopril tablets 10 mg, 20 mg tablets correspond to the USP XXIV requirements: 10 mg ± 10 %; 20 mg ± 10 % (USP XXIV, 2000).

The analytical parameters repeatability (precision) and accuracy are determined by the uncertainty of the result, which relevantly for Lisinopril tablets 10 mg, 20 mg (table 2) and for model mixtures I, II, III (L5, L10, L15) (Table 3), is presented by standard deviations (SD), relative standard deviations (RSD) and confidential interval ($\overline{X} \div t \cdot S\overline{X}$). All of the experimental data suit confidential intervals. The accuracy is determined by the degree of recovery (R) (Dimov, 1999).

Conclusion

HPLC analytical parameters for the examined tablets correspond to the USP XXIV requirements. The experimental results from the content of Lisinopril in Lisinopril tablets 10 mg, 20 mg suit USP XXIV requirements: 10 mg ± 10 % (9 mg ÷ 11 mg); 20 mg ± 10 %

(18 mg ÷ 22 mg). The HPLC method for quantity analysis of Lisinopril in tablets is validated by the obtained data for precision and accuracy and is confirmed, that this method can be applied for quality control of different trade drug products – tablets, containing Lisinopril.

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