FOSINOPRIL–SODIUM AND ITS DEGRADATION PRODUCT ANALYSIS IN MONOPRIL® TABLETS†

Biljana Jančić,[a] Darko Ivanović,[a] Mirjana Medenica,[b] and Andjelija Malenović[a]

[a] *Institute of Drug Analysis, Faculty of Pharmacy, Vojvode Stepe 450, 11000 Belgrade, Yugoslavia*
[b] *Institute of Physical Chemistry, Faculty of Pharmacy, Vojvode Stepe 450, 11000 Belgrade, Yugoslavia*

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Abstract

The reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed for the simultaneous determination of fosinopril–sodium and its degradation product in Monopril® tablets. The chromatographic system Hewlett Packard 1100 consisted of a HP 1100 pump, HP 1100 UV-VIS detector and HP ChemStation integrator. Separations were performed on the X Terra™ 150 mm x 4.6 mm, 5 μm particle column at 45 °C. The samples were introduced through a Rheodyne injector valve with a 20 μL sample loop. Methanol–water (75 : 25 V/V) was used as a mobile phase, with flow rate 1 mL/min. pH was adjusted to 3.1 with ortophosphoric acid. UV detection was performed at 220 nm. Propylparaben was used as an internal standard. The results obtained showed a good agreement with the declared contents. Recovery values for fosinopril–sodium were from 101.6% to 102.9%. Content of degradation product SQ 27519 was lower than 5%. The proposed method is rapid, accurate, selective and because of its sensitivity and reproducibility, it may be used for the quantitative analysis of fosinopril–sodium and its degradation product in Monopril® tablets.

Introduction

Fosinopril, a phosphinic acid derivative, is an angiotensin converting enzyme (ACE, bradykininase, kininaze II) inhibitor. Fosinopril is a prodrug and hydrolyses to the active diacid product, named SQ 27519.[1] It differs from other ACE inhibitors by the presence of a phosphinic acid group. Fosinopril-sodium is the active agent of Monopril® tablets. It is not official substance in any Pharmacopeia and the only references concerning this substance and its degradation product in diacid form SQ 27519 (in the following text DP) were found in a few papers.[1,2]

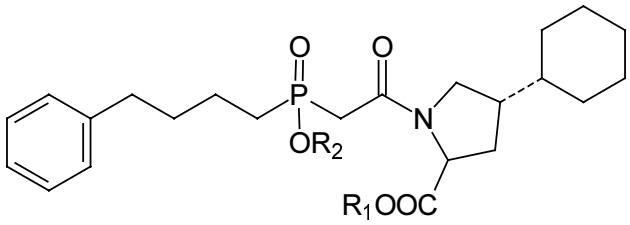
Monopril® tablets contain 10 mg of fosinopril–sodium (sodium [1[S*(R*)], 2α, 4β]-4-cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy)propoxy](4-phenylbutyl)phosphinyl]-

acetyl]-L-proline)[2] and not more than 5% of its degradation product (DP), calculated to the content of fosinopril–sodium. The reversed–phase high–performance liquid chromatographic (RP–HPLC) method has been applied for the simultaneous determination of fosinopril–sodium and DP. Active substance is an ester and its degradation product is the diacid, so the aim of the investigation was to develop RP–HPLC method which makes possible simultaneous determination of mixture of substances with so similar structure but different polarity and to investigate the linearity, precision, limit of quantitation and limit of detection.

There are a few references of fosinopril and some papers described the combination of fosinopril–sodium and hydrochlorotiazide in tablets which was analysed by RP–HPLC method,[3] by spectrophotometric method[4] or derivative spectrophotometric and RP–HPLC method.[5]

Results and discussion

Chemical structure and physico–chemical characteristics are the most important facts that predict chromatographic behavior. Fosinopril–sodium is the ester and after degradation the diacid product named SQ 27519 is formed (Chart).



COMPOUND	R_1	R_2
Fosinopril–sodium	Na	$(CH_3)_2-CH-\underset{\displaystyle O}{\overset{\displaystyle \|}{C}}-C_2H_5$
SQ 27519	H	H

Chart

In the presented investigation the best separation of this two compounds was achieved using XTerra™ column 150×4.6 mm particle size 5 μm. Using the other C_{18} column 150×4.6 mm, 5 μm particle size under the same experimental conditions, the

separated lasted about 30 minutes. XTerra^{TM} column differs from the other C_{18} columns by methyl groups attached to free silanol groups – encapped column. Duration of the separation is shorter and peak symmetry better. For the separation and determination of fosinopril–sodium and DP in Monopril® tablets the best results were obtained using mobile phase methanol–water (75:25 V/V). The lower percentage of methanol in mobile phase results in peak tailing of both components and long analysis duration. Optimal retention times (fosinopril–sodium – 7.026 min and DP – 4.835 min) were achieved when the pH of the mobile phase was adjusted to 3.1 with 85% ortophosphoric acid. Small changes in pH of the mobile phase had a greate influence to the chromatographic behavior of these substances. At a lower pH, retention times of fosinopril–sodium and DP are extremely long, but higher pH of mobile phase result in peak tailing. Representative chromatograms of laboratory mixture and Monopril® tablets are given in Figure 1.

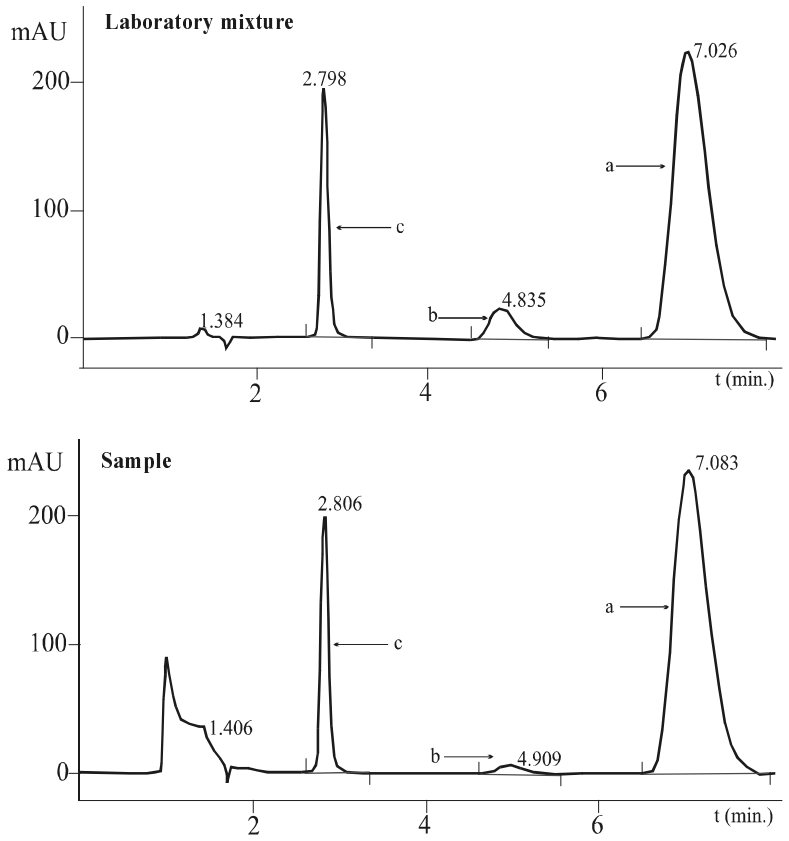


Figure 1. The representative chromatogram of fosinopril-sodium (a), degradation product (b) and internal standard (c).

After establishing the optimal conditions for the separation, linearity, precision, limit of quantitation and limit of detection were investigated.

A linear relationships of the peak area (y), e.g. area ratios of samples and internal standard over the mentioned concentration (c) ranges for fosinopril–sodium and DP were obtained. The important calibration curve parameters: slope (*a*), intercept (*b*), correlation coefficient (*r*) and standard deviation of the intercept (S_b) are presented in Table 1.

Table 1. The important parameters for the calibration curves.

Compound	y = ax + b	r	S_b
Fosinopril–sodium	10375x + 75.88 (x (=) mg/mL)	0.9997	57.943
Degradation product	14.0 x + 7.43 (x (=) μg/mL)	0.9999	2.91

y – peak area; x – concentration; a – slope; b – intercept;
r – correlation coefficient; S_b – standard deviation of the intercept.

Limit of detection (*LOD*) and limit of quantitation (*LOQ*) were experimentally determined and they are presented in Table 2, as well as coefficient of variation (CV) for detector response factor.

Table 2. Some important parameters.

Compound	LOQ* (μg/mL)	LOD* (μg/ml)	CV** (%)
Fosinopril–sodium	6.0	1.0	2.1
Degradation product	1.5	0.2	3.7

*Experimentally determined values; LOQ–limit of quantitation; LOD–limit of determination; **CV–coefficient of variation for detector response factor.

The best resolution was obtained using propylparaben as an internal standard. The concentrations of fosinopril–sodium and degradation product were calculated by the internal standard method.

The results of precision of RP–HPLC determination are given in Table 3. The important statistical values, such as standard deviation (S), coefficient of variation (CV) and statistical parameter t_α, as well as good recoveries, are given in Table 3. Standard deviation and coefficient of variation have a small required values. As the

experimentally determined values of t_α are lower than $t_{tab.}$ ($p = 0.01$), precision of developed RP-HPLC method was confirmed.

The results for the determination of content of fosinopril–sodium and degradation product in Monopril® tablets are are given in Table 4. The important statistical values, such as standard deviation and coefficient of variation, as well as good recoveries, are given in Table 4. Standard deviation and coefficient of variation have a small required values. Monopril® tablets (produced in 2001) were analysed. Tablets were stored at the room temperature as it is stated at the declaration. The content of DP is less than 5%, so a good quality of tablets is confirmed (Table 4).

Table 3. Precision of the Monopril® tablets assay.

Compound	Injected	Found	CV (%)	R (%)	t_α
Fosinopril–sodium (mg/mL)	0.4	0.407 ± 0.003*	0.7	101.6	2.56
	0.6	0.611 ± 0.003	0.5	101.9	2.36
	0.8	0.822 ± 0.007	0.9	102.7	2.85
Degradation product (μg/mL)	20	20.1 ± 0.3*	1.5	100.5	2.70
	30	30.9 ± 0.2	0.7	103.1	2.72
	40	41.7± 0.3	0.7	104.3	2.48

*S (n=10); $t_{tab.}$ = 3.250 ($p = 0.01$).

Table 4. Monopril® tablets determination.

Compound	Taken (mg/mL)	Found (mg/mL)	Found (mg/tbl.)	CV (%)	R (%)
Fosinopril–sodium	0.4	0.407 ± 0.004*	10.17 ± 0.10*	1.0	101.6
	0.6	0.615 ± 0.006	10.26 ± 0.09	0.9	102.5
	0.8	0.823 ± 0.003	10.29 ± 0.04	0.4	102.9

*S (n=10)

Compound	Taken (μg/mL)	Found (μg/mL)	Found (mg/tbl.)	Found (%)	CV (%)
Degradation product	20	2.40 ± 0.09*	0.059 ± 0.002*	0.71	3.4
	30	3.82 ± 0.09	0.062 ± 0.002	0.74	3.2
	40	7.09 ± 0.03	0.066 ± 0.002	0.79	3.0

*S (n=10)

Conclusions

The proposed RP-HPLC method enables simultaneous determination of fosinopril–sodium and his degradation product because of a good separation and resolution of the chromatographic peaks. Method is applicable for a qualitative and quantitative analysis of the Monopril® tablets. The obtained results are in a good agreement with the declared contents. Results are accurate and precise and confirmed by statistical parameters. There was no interference of the excipient in tablets. The proposed method is rapid, precise and it estimates determination of fosinopril–sodium indipendently of the degradation product.

Experimental

Chemicals. All reagents used were of an analytical grade. Methanol – gradient grade (*Merck*, Darmstadt, Germany), HPLC grade water and 85% ortophosphoric acid (*Carlo Erba*, Milan, Italy) were used to prepare a mobile phase. Monopril® tablets (contain 10 mg of fosinopril–sodium) was manufactured by *Bristol–Myers Squibb*, as well as the working standards of fosinopril-sodium and degradation product.

Chromatographic conditions. The chromatographic system Hewlett Packard 1100 consisted of HP 1100 pump, HP 1100 UV–VIS detector and HP ChemStation integrator. Separations were performed on a X–Terra™ 4.6 mm x 150 mm, 5μm particle size column at 45 °C. The samples were introduced through a Rheodyne injector valve with a 20 μL sample loop.

Separation and simultaneous determination of fosinopril–sodium and degradataion product were performed using the mobile phase methanol–water (75:25 V/V). pH was adjusted to 3.1 with 85% ortophosphoric acid. Mobile phase was filtered through a 0.2 μm Millipore filter and degassed in an ultrasonic bath. Propylparaben was used as an internal standard. The flow rate of the mobile phase was 1 mL/min and UV detection was performed at 220 nm.

Standard solutions. Concentration ranges of standard solutions for calibration curves were from 0.05 mg/mL to 1.0 mg/mL for fosinopril–sodium and from 2 μg/mL to 50 μg/mL for SQ 27519.

Laboratory mixtures. Laboratory mixtures which corresponded to Monopril® tablets were prepared of fosinopril–sodium (0.4 mg/mL, 0.6 mg/mL and 0.8 mg/mL) and

SQ 27519 (20 μg/mL, 30 μg/mL and 40 μg/mL). For the chromatographic separation propylparaben was added as an internal standard in concentration of 40 μg/mL.

Sample solutions. Twenty Monopril® tablets were accurately weighted and finally powdered. That amount was transferred with 70 mL of mobile phase to a 100 mL volumetric flask and dissolved in an ultrasonic bath for 20 min. The volumetric flask was filled to the mark with mobile phase and the resulting solution was filtered. The concentration of fosinopril–sodium in this solution was 2.0 mg/mL. The diluted solutions were prepared in the same concentrations as laboratory mixture. Propylparaben was added as an internal standard (40 μg/mL). Resulting solutions were injected in the column.

References and Notes

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Povzetek

Razvili smo reverzno-fazno metodo tekočinske kromatografije visoke ločljivosti (RP-HPLC) za simultano določanje fozinopril-natrija in njegovega razpadnega produkta v Monopril® tabletah. Uporabili smo kromatografski sistem Hewlett Packard 1100, ki je bil sestavljen iz črpalke HP 1100, HP 1100 UV-VIS detektorja in HP ChemStation integratorja. Separacije smo izvajali na koloni X Terra™ dimenzij 150 mm x 4,6 mm s 5 μm delci pri temperaturi 45 °C. Za nanašanje vzorcev smo uporabili Rheodyne injektor z 20 μl zanko. Uporabili smo mobilno fazo sestavljeno iz metanola in vode v razmerju 75:25 (V/V) pri pretoku 1 mL/min. Z ortofosforjevo kislino smo uravnali pH na 3,1. Analize smo izvajali z UV detekcijo pri 220 nm, pri čemer smo kot interni standard uporabili propilparaben. Dobljeni rezultati kažejo na dobro skladnost z deklarirano vsebnostjo. Izkoristki fozinopril-natrija so bili v območju med 101,6% in 102,9%. Vsebnost razpadnega produkta SQ 27519 je bila pod 5%. Predlagana metoda je hitra, točna in selektivna ter se lahko zaradi svoje občutljivosti in reproducibilnosti uporablja za kvantitativno analizo fozinopril-natrija in njegovega razpadnega produkta v Monopril® tabletah.