CONGRESUL CONSACRAT ANIVERSĂRII A 75-A DE LA FONDAREA USMF „NICOLAE TESTEMIȚANU”
20 23 octombrie 2020

DEVELOPMENT AND VALIDATION OF THE HIGH-PRESSURE LIQUID CHROMATOGRAPHY METHOD FOR PROPYLTIOHINOTIADIAZOL DOSAGE

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INTRODUCTION

Propylthiohinothiadiazole is a thiadiazole derivative with pronounced antimycobacterial action. Substance is researched in order to develop methods of analysis and standardization, analytical documents regulating quality for use as an active principle in the development of pharmaceutical forms.

The purpose. Elaboration of the work technique and validation of the HPLC method for dosing propylthiohinothiadiazole.

MATERIAL AND METHODS

3 series of propylthiohinothiadiazole, internal reference standard of 2-(propylthio)-5H-[1,3,4]-thiadiazole[2,3-b]-quinazolin-5-one substance with concentration 99,98%, chromatographic system Shimadzu LC-20AD HPLC with UV-VIS detector, acetonitrile and dimethylsulfoxide of purity grade “pro HPLC analysis” (Sigma Aldrih and Merck), bidistilled purified water.

RESULTS

Linearity is proved for concentrations of 5-30 µg/ml, the linear regression equation is y=36472x-44580, R^2=0.9929. The limit of detection is 0,729 µg/ml and the limit of quantification is 2,210 µg/ml. It was established that the method is accurate (mean recovery values at 80%, 100% and 120% concentration levels were close to 100%. The accuracy of the method was expressed by repeatability and intermediate accuracy. The variation of the chromatographic conditions established that the method is robust. For all validation parameters, RSD was less than 1.

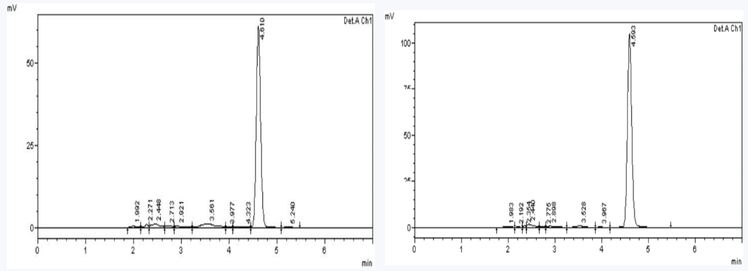

Fig. 1. Chromatograms of propylthiohinothiadiazole solutions using for linearity determination

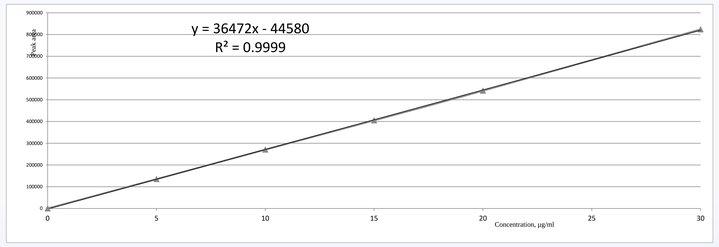

Fig. 2. Calibration curve of standard propylthiohinothiadiazole solution

Table 1. Results of accuracy of developed method of assay of propylthiohinothiadiazole

Concentration levels, %	Number of determinations	Theoretical concentration of spiked sample, µg/ml	Peak area	Concentration in spiked sample, µg/ml	% recovery	Average, % RSD
80	1	8	6746764.55	8.045	100.56	100.530
	2	8	6747213.28	8.046	100.58	
	3	8	6739989.94	8.037	100.46	0.061
100	1	10	8433455.69	10.045	100.45	100.710
	2	10	8460275.02	10.077	100.77	
	3	10	8472697.44	10.091	100.91	0.240
120	1	12	10110546.81	12.033	100.28	100.260
	2	12	10108824.57	12.031	100.26	
	3	12	10105489.14	12.027	100.23	0.031

Note: RSD – relative standard deviation

Table 2. Results of determinations of repeatability of the HPLC method for propylthiohinothiadiazole assay

No.	Retention time, min	Peak area	Assay, %
1	4.483	8433455.69	100.45
2	4.487	8460275.02	100.77
3	4.471	8472697.44	100.91
4	4.477	8439984.05	100.53
5	4.479	8433455.69	100.45
6	4.484	8439984.05	100.53
Average	4.480	8446641.98	100.60
RSD, %	0.128	0.191	0.188

Table 3. Results of intermediate precision determinations in the validation of the propylthiohinothiadiazole HPLC method

	Day 1			Day 2		
No.	Retention time, min	Peak area	Assay, %	Retention time, min	Peak area	Assay, %
1	4.483	8433455.69	100.45	4.495	9275724.35	101.73
2	4.487	8460275.02	100.77	4.937	9303352.96	102.03
3	4.471	8472697.44	100.91	4.922	9189046.32	100.78
4	4.477	8439984.05	100.53	4.956	9388533.41	102.96
5	4.479	8433455.69	100.45	4.988	9307879.46	102.08
6	4.484	8439984.05	100.53	4.943	9268835.80	101.65
Average	4.480	8446641.98	100.60	4.949	9288895.38	101.87
RSD, %	0.128	0.191	0.188	0.450	0.698	0.698

Table 4. Results of the determination of the robustness of the HPLC method for dosing propylthiohinothiadiazole

Variable parameters		Retention time min	Peak area	Assay, %
Mobile phase flow 0.9 ml/min		4.482	8434755.24	99.72
Mobile phase flow1,1 ml/min		4.472	8461005.87	100.03
Acetonitrile: purified water 80:20		4.486	8473097.57	100.17
Acetonitrile: purified water81.6:18.4		4.479	8439784.07	99.78
Column temperature 30^0 C		4.481	8432915.21	99.70
Column temperature 35^0 C		4.482	8438584.13	99.77
The average value		4.480	8.45E+06	99.86
Standard deviation	S^2	2.19E-05	2.70E+08	3.77E-02
	S	0.005	16418.795	0.194
Relative standard deviation	RSD	0.104	0.194	0.194

Table 5. Results of solution stability testing for HPLC propylthiohinothiadiazole assay method

Determined parameters	Storage period	Retention time min	Peak area	Assay, %	RSD for the peak area
Standard solution	0 h	4.483	8457894.12	-	0.03
	24 h at 25^{0}C	4.479	8457387.08	100.09	0.17
	24 h at 4^{0}C	4.482	8457614.21	100.10	0.07
Sample solution	0 h	4.484	8434755.24		0.05
	24 h at 25^{0}C	4.484	8432915.21	99.70	0.21
	24 h at 4^{0}C	4.479	8433089.57	99.71	0.11

CONCLUSIONS

The validation results show that the developed HPLC method is simple, fast, accurate and reproducible.

Keywords: HPLC, propylthiohinothiadiazole, dosing, validation.

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