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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULATANEOUS ESTIMATION OF PYRANTEL PAMOATE AND ALBENDAZOLE IN BULK AND ITS TABLET DOSAGE FORM

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ABSTRACT

The purpose of the investigation was to develop a new RP-HPLC Method for simultaneous estimation of Pyrantel pamoate and Albendazole in pharmaceutical dosage forms. Chromatography was carried out on an BDS C-18 column (4.6 x 250mm, 5µ particle size) with a isocratic mobile phase composed of 0.1% Potassium dihyrogen Ortho phosphate (adjusted to pH 4.8 with triethylamine solution), Acetonitrile, methanol (40:40:20v/v) at a flow rate of 1 mL/min. The column temperature was maintained at 30°C and the detection was carried out using a PDA detector at 311 nm. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), Stability of sample & standard stock solutions, robustness AND degradation studies were determined as reported in the International Conference on Harmonization guidelines. The retention times for Pyrantel pamoate and Albendazole and were 2.161 min and 3.405 min respectively. The percentage recoveries of Pyrantel pamoate and Albendazole were 99.29% and 99.40% respectively. The relative standard deviation for assay of tablets was found to be less than 2%. The Method was fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in quality control laboratories and pharmaceutical industries.

Keywords: Pyrantel pamoate, Albendazole, Simultaneous estimation, ICH guidelines.

INTRODUCTION

Pyrantel pamoate (ACB) is chemically named as 4-[(3-Carboxy-2-hydroxynaphthalen-1-yl)methyl]-3-hydroxynaphthalene-2-carboxylic acid; 1-methyl-2-[(E)-2-thiophen-2-ylethenyl]-5,6-dihydro-4H-pyrimidine. It is used as a deworming agent in the treatment of hookworms (all species) and roundworms (Ascaris lumbricoides, aka ascarids in humans) in domesticated animals such as horses, cattle, sheep, pigs, cats, dogs, and many other species[1]. Albendazole is chemically named as methyl N-[6-(propylsulfanyl)-1H-1,3-benzodiazol-2-yl]carbamate. Albendazole causes degenerative alterations in the tegument and intestinal cells of the worm by binding to the colchicine-sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules[2].

Various UV & HPLC assay Methods are also reported in the literature for the estimation of Pyrantel pamoate[3-4] and Albendazole [5-9] individually and in-combination with other drugs. According to literature survey there is no official Method for the simultaneous estimation of Pyrantel pamoate and Albendazole by RP-HPLC in combined tablet dosage forms. Hence, an attempt has been made to develop new Method for simultaneous estimation and validation of Pyrantel pamoate and Albendazole in tablet formulation in accordance with the ICH guidelines[10-12].

EXPERIMENTAL

Instrumentation: Chromatography was performed with Alliance waters 2695 HPLC provided with high speed auto sampler, column oven, degasser and &

2996 PDA detector to provide a compact and with class Empower-2 software.

Reagents and chemicals: The reference samples of Pyrantel pamoate and Albendazole were provided as gift samples from Spectrum pharma research solutions, Hyderabad. HPLC grade acetonitrile, HPLC grade mehanol and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Commercial tablets (Wormazan ; Dosage: Pyrantel Pamoate -100 mg & Albendazole- 300 mg) were purchased from the local pharmacy.

Chromatographic condition: The chromatographic separation was carried out under the isocratic conditions. Chromatographic separation was achieved by injecting a volume of 10µl of standard into BDS (250 x 4.6 mm, 5µ) column. The mobile phase composed of 0.1% Potassium dihyrogen Ortho phosphate (adjusted to pH 4.8 with triethylamine solution), Acetonitrile, mehanol (40:40:20v/v) was allowed to flow through the column at a flow rate of 1 ml per minute for a period of 7 min at 30^0C column temperature. Detection of the component was carried out at a wavelength of 311 nm. The retention time of the components were found to be 2.161 min and 3.405 min for Pyrantel pamoate and Albendazole respectively.

Preparation of diluent solution: Diluent solution was prepared by mixing 500 ml of HPLC grade water with 500ml of mehanol, in a 1000ml beaker and sonicated for 15min.

Preparation of standard stock solution: Accurately Weighed and transferred 10mg& 30mg of Pyrantel Pamoate and Albendazole working Standards into 25ml clean dry volumetric flasks, add $3/4^{th}$ vol of diluent, sonicated for 30 minutes and make up to the final volume with diluents.From the above stock solution, 1 ml was pipeted out in to a 10ml volumetric flask and then make up to the final volume with diluent.

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***Preparation of Working Standard Solutions*:** Aliquot of 0.25, 0.50, 0.75, 1, 1.25 & 1.5 mL were pipette out from stock solution into 10 mL volumetric flask separately for both Pyrantel Pamoate and Albendazole and volume was made up to 10 mL with diluent. This gives the solutions of 10, 20, 30, 40, 50and 60µg/mL for Pyrantel pamoate and 30, 60, 90, 120, 150and 180µg/mL for Albendazole respectively.

Sample preparation: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 250mL volumetric flask, 200mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made upto 10ml with diluent.

Method validation:

System suitability tests: To ensure the resolution and reproducibility of the HPLC system was adequate for the analysis, a system suitability test was established. Data from six injections of 10 µL of the working standard solutions of Pyrantel Pamoate and Albendazole were used for the evaluation of the system suitability parameters like tailing factor, the number of theoretical plates, retention time and resolution factor.

Linearity: By appropriate aliquots of the standard pyrantel pamoate and albendazole solutions with the mobile phase, six working solutions ranging between 10-60 µg/mL and 30-180 µg/mL were prepared. Each experiment linearity point was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of pyrantel pamoate and albendazole to obtain the calibration curve.

Accuracy: Recovery studies by the standard addition Method were performed with a view to justify the accuracy of the proposed Method. Previously analyzed samples of pyrantel pamoate and albendazole to which known amounts of standard pyrantel pamoate and albendazole corresponding to 50%, 100% and 150% of target concentration were added. The accuracy was expressed as the percentage of analyte recovered by the proposed Albendazole.

Precision: Precision was determined as repeatability and intermediate precision (ruggedness), in accordance with ICH guidelines. The intra-day and inter-day precision were determined by analyzing the samples of Pyrantel Pamoate and Albendazole. Determinations were performed on the same day as well as well as on consequent days.

Limit of detection and the limit of quantification: Limit of detection (LOD) and limit of quantification (LOQ) of pyrantel pamoate and albendazole were determined by calibration curve. Solutions of both pyrantel pamoate and albendazole were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by

using following equations. LOD = (3.3 ×Syx)/b, LOQ= (10.0×Syx)/b
Where Syx is residual variance due to regression; b is slope.

Robustness: The robustness of pyrantel pamoate and albendazole was performed by deliberately changing the chromatographic conditions. The organic strength was varied by ±5%, column temperature was varied by $\pm 5^0$c and the flow rate was varied by ±0.1mL.

Stability: The sample and standard solutions injected at 0 hr (comparison sample) and after 24 hr (stability sample) by keeping at ambient room temperature. Stability was determined by determining %RSD for sample and standard solutions.

Degradation studies:

Oxidation: To 1 ml of stock solution of Albendazole and Pyrantel pamoate, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 40 µg/mL Pyrantel Pamoate & 120 µg/mL Albendazole solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of stock solution Albendazole and Pyrantel pamoate, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 40 µg/mL Pyrantel Pamoate & 120 µg/mL Albendazole solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies: To 1 ml of stock solution Albendazole and Pyrantel pamoate, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 40 µg/mL Pyrantel Pamoate & 120 µg/mL Albendazole solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105 °C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 40 µg/mL Pyrantel Pamoate& 120 µg/mL Albendazole solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the 80µg/ml&100µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 40 µg/mL Pyrantel Pamoate& 120 µg/mL Albendazole solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 40 µg/mL Pyrantel Pamoate& 120 µg/mL Albendazole solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

RESULTS AND DISCUSSION:

pyrantel pamoate and albendazole method development:
Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol and Water, Acetonitrile and Water as mobile phases, in which both the drugs did not responded properly, and the resolution was also poor. The organic content of mobile phase was also investigated to optimize the separation of both drugs. To improve the tailing factor, the pH of mobile phase becomes important factor. Thereafter, 0.1% Potassium dihyrogen Ortho phosphate buffer (adjusted to pH 4.8 with dil. Orthophosphoric acid solution): acetonitrile were taken in isocratic ratio: 50: 50 and with flow rate of 1.0 mL/min was employed. BDS column (4.6 x250mm, 5µ particle size) was selected as the stationary phase to improve resolution and the tailing of both peaks were reduced considerably and brought close to 1. To analyze both drugs detection were tried at various wavelengths from 205nm to 280nm. Both pyrantel pamoate and albendazole showed maximum absorption at 311 nm of wavelength and 311 nm was selected as the detection wavelength for PDA detector. The retention times were found to about 2.16 min and 3.4 min for pyrantel pamoate and albendazole, respectively. The chromatogram obtained was shown in the Fig. 2.

pyrantel pamoate and albendazole method Validation:
System suitability and Specificity: System suitability parameters such as number of theoretical plates, peak tailing, and retention time and resolution factor were determined. The total run time required for the

method is only 6 minutes for eluting both pyrantel pamoate and albendazole. The results obtained were shown in Table No.1.

Linearity: pyrantel pamoate showed a linearity of response between 10-60 µg/mL and albendazole showed a linearity of response between 30-180 µg/mL. These were represented by a linear regression equation as follows: y (pyrantel pamoate) = 62185x + 290.46 (r^2=0.999), y(albendazole)= 31867x + 571.29 (r2=0.999) and regression line was established by least sqsuares method and correlation coefficient (r^2) for pyrantel pamoate and albendazole is found to be greater than 0.98. Hence the curves established were linear.

Accuracy: To pre analyzed sample solution, a definite concentration of standard drug (50%, 100% & 150 % level) was added and recovery was studied. The % Mean recovery for pyrantel pamoate and albendazole are 100.00 and 100.05 respectively and these results are within acceptable limit of 98-102. The % RSD for pyrantel pamoate and albendazole are 0.50 and 0.20 respectively and %RSD for pyrantel pamoate and albendazole is within limit of ≤2, hence the proposed method is accurate and the results were summarized in Table No.2.

Precision: Repeatability: Six replicates injections in same concentration (40µg/ml,120µg/ml of pyrantel pamoate and albendazole respectively) were analyzed in the same day for repeatability and the % RSD for pyrantel pamoate and albendazole found to be 0.47 and 0.25 respectively and % RSD for pyrantel pamoate and albendazole found to be within acceptable limit of ≤2 and hence method is reproducible and the results are shown in Table No. 3**.**

Intermediate Precision: Six replicates injections in same concentration were analyzed on two different days with different analyst and column for verifying the variation in the precision and the % RSD for pyrantel pamoate and albendazole is found to be 1.4 and 0.5 and it is within acceptable limit of ≤2. Hence the Method is reproducible on different days with different analyst and column. This indicates that the method is precise and the results are as shown in Table No. 3.

Robustness: The robustness was established by changing the flow rate, column temperature and composition of the mobile phase within allowable limits from actual chromatographic conditions. It was observed that there were no marked change in mean R_t and RSD is within limit of ≤2 .The tailing factor, resolution factor and no. of theoretical plates are found to be acceptable limits for both pyrantel pamoate and albendazole. Hence the Method is reliable with variations in the analytical conditions and the results of pyrantel pamoate are shown in Table No.4 and results of albendazole shown in Table No. 5.

Stability of sample solution:. The sample solution injected after 24 hr by keeping at ambient room temperature 30^{O}C did not show any appreciable change. The % Deviation in the assay is not more than 2 and the results are shown in table-6.

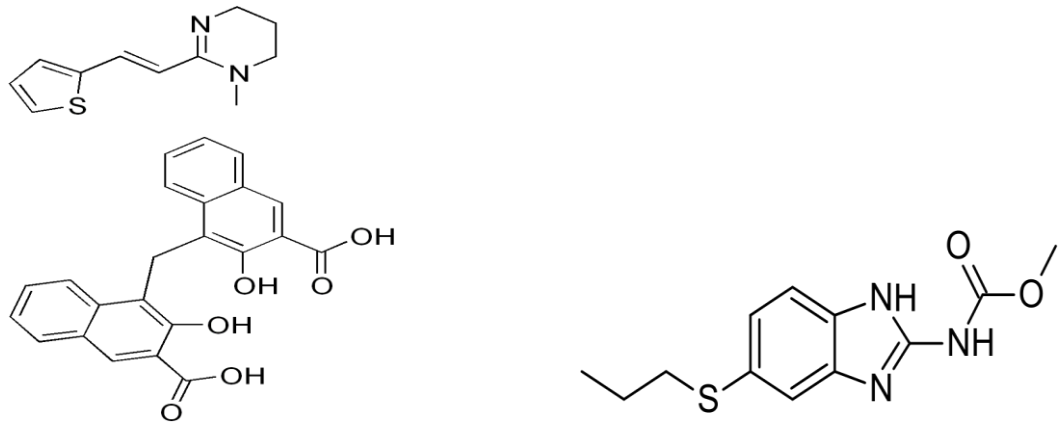
LOD and LOQ: LOD and LOQ for pyrantel pamoate were 0.02 and 0.05 µg/mL respectively and for albendazole were 0.03 and 0.09 µg/mL, respectively. The lowest values of LOD and LOQ as obtained by the proposed Method indicate that the Method is sensitive.

Tablet Analysis: The Content of pyrantel pamoate and albendazole in the tablets was found by the proposed method. RSD values for both pyrantel pamoate and albendazole are within limit of ≤2 and the results were shown in Table No. 7.

Stability Studies: The results for degradation in oxidative, acidic, alkaline, dry heat, photo stability and neutral degradation were showed in table 8 and 9.

CONCLUSION:

A new precise accurate and simple stability indicating HPLC Method was developed and validated for simultaneous estimation of Pyrantel pamoate and Albendazole tablet dosage form. This Method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in QC laboratories and industries.



Pyrantel pamoate **Albendazole**

Fig.1.Chemical structures of drugs investigated in this study

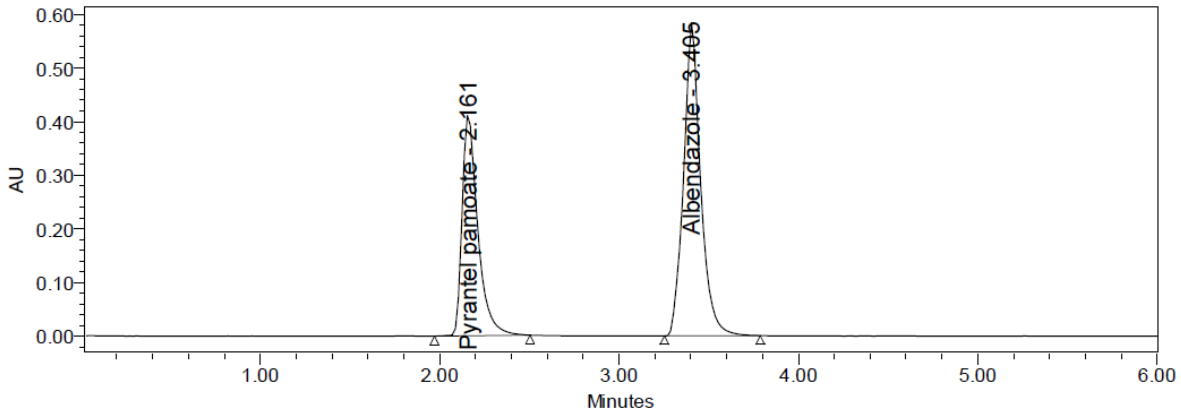


Fig.2.A typical Chromatogram of Pyrantel Pamoate and Albendazole

Table No.1: System suitability of Pyrantel Pamoate and Albendazole

SYSTEMSUITABILITY PARAMETERS	PYRANTEL	ALBENDAZOLE
No of theoretical plates	3189	5697
Tailing Factor	1.58	1.35
Resolution	-	7.22
RT	2.1 min	3.4 min
Mean Area	2496761	3830518
%RSD	0.4	0.4

Table No.2: Results of accuracy of Pyrantel Pamoate and Albendazole

Sample	Amount Taken (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Pyrantal pamoate	20	20.02	100.02	0.69
	40	39.82	99.55	0.09
	60	60.25	100.43	0.7
Albendazole	60	60.00	100.00	0.7
	120	119.95	99.96	0.93
	180	180.37	100.21	0.89

Table No.3: Results of Precision for Pyrantel and Albendazole

Repeatability data for pyrantal pamoate and Albendazole

Sr. No.	Pyrantal pamoate	Albendazole
1	2481788	3825610
2	2480462	3812223
3	2464817	3821164
4	2496938	3834450
5	2484498	3811690
6	2495734	3831244
Mean	2484040	3822730
Std. Dev.	11753.4	9517.1
%RSD	0.5	0.2

Inter day precision results for pyrantal pamoate and Albendazole

Sr. No.	Pyrantal pamoate	Albendazole
1	2417027	3755823
2	2401571	3752605
3	2425004	3770682
4	2451074	3716510
5	2438827	3733155
6	2497770	3731762
Mean	2438546	3743423
Std. Dev.	33699.0	19732.4
%RSD	1.4	0.5

Table No. 4: Results of Robustness for Pyrantel Pamoate

Analytical conditions Evaluation paraAlbendazoleers	Flow rate (ml/min)		Column temperature (^{o}c)		Mobile phase composition	
	1.2	1.0	35	25	+5%	-5%
Mean RT	2.164	2.163	2.046	2.166	2.150	2.165
Mean area	2493139	2520834	2496652	2522379	2491310	2488225
SD	2570.0	9562.9	943.5	20500	10569	7484
RSD	0.1	0.37	0.03	0.8	0.42	0.30
Tailing factor	1.50	1.52	1.58	1.49	1.51	1.51
No. of theoretical plates	3328	3212	3298	3470	3300	3470

Table 5 : Results of Robustness for Albendazole

Analytical conditions Evaluation parameters	Flow rate(1.0 ml/min)		Column temperature		Mobile phase composition	
	1.2	1.0	35	25	+5%	-5%
Mean RT	3.421	3.418	3.234	3.420	3.234	3.420
Mean area	3828553	3811819	3821546	3828559	3844181	3844175
SD	8731.0	13023.5	15751	8737.5	6884	6890.5
RSD	0.22	0.34	0.41	0.22	0.17	0.17
Tailing factor	1.05	1.06	1.03	1.05	1.03	1.05
No. of theoretical plates	9782	8041	8874	11867	8871	11869

Table 6: Results of stock solution stability for Pyrantel Pamoate and Albendazole

Drug	%Assay at 0 hr	%Assay at 24 hr	%Devaition
PYRANTEL PAMOATE	99.29	99.17	0.12
ALBENDAZOLE	99.40	100.16	0.76

Table 7: Results of HPLC Analysis of Tablet for Pyrantel Pamoate and Albendazole

Label amount (mg)		Amount found(mg) n=6		%Assay (Mean±SD)		RSD	
pyrantel pamoate	albendazole	pyrantel pamoate	albendazole	pyrantel pamoate	albendazole	pyrantel pamoate	albendazole
50	500	50.09	500.5	100.90±1.098	100.25±1.002	0.5	0.5

Table 8 Degradation Data of Albendazole

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	3.89	0.161	0.297
2	Alkali	3.63	0.178	0.290
3	Oxidation	3.54	0.164	0.296
4	Thermal	2.28	0.249	0.306
5	UV	1.65	0.184	0.291
6	Water	0.88	0.279	0.291

Table 9 Degradation Data of Pyrantel pamoate

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.25	0.239	0.245
2	Alkali	1.2	0.220	0.241
3	Oxidation	2.5	0.242	0.244
4	Thermal	1.42	0.228	0.248
5	UV	1.46	0.223	0.242
6	Water	0.52	0.222	0.242

REFERENCES:

1. Validation of Analytical Procedures, ICH Harmonised Tripartite Guidelines, Q2B, 1997.
2. Synder LC., Kirkland JJ and Glajch JL,Practical HPLC Method Development, 2nd ed., John Wiley & Sons, 1997;210-556.
3. Sethi P, HPLC Quantitative Analysis of Pharmaceutical Formulations, 1st ed., CBS Publication and Distributor, 2001; 14-15.
4. Mahler, Graziela, Davyt, D., Gordon, Sandra, Incerti, M., Nunez, Ivana, Pezaroglo, H., Scarone, Laura, Serra, Gloria, Silvera, M., de Manta, E., (2008).Synthesis of lbendazole Metabolites: Characterization and HPLC Determination. J. Chem. Educ., 85(12), 1652.
5. M.I. Yasawy, M.A.A.L. Karawi, A.R.E. Mohamed. Combination of praziquantel and albendazole in the treatment of hydatid disease.Trop. Med. Parasitol., 44 (1993), pp. 192–194
6. F. Cobo, C. Yarnoz, B. Sesma, P. Fraile, M. Aizcorbe, R. Trujillo, A. Diazde- Liaño, M.A. Ciga. Albendazole plus praziquantel versus albendazole alone as a pre-operative treatment in intra-abdominal hydatidosis caused by *Echinococcus granulosus.* Trop. Med. Int. Health, 3 (1998), pp. 462–466.
7. A.E. Mohamed, M.I. Yasawy, M.A. Al Karawi. Combined albendazole and praziquantel versus albendazole alone in the treatment of hydatid disease. Hepatogastroenterology, 45 (1998), pp. 1690–1694
8. H.M. Ayles, E.L. Corbett, I. Taylor, A.G.A. Cowie, J. Bligh, K. Walmsley, A.D.M. Bryceson. A combined medical and surgical approach to hydatid disease: 12 years' experience at the Hospital for Tropical Diseases. Ann. R. Coll. Surg. England, 84 (2002), pp. 100–105.
9. C. Villaverde, A.I. Alvarez, P. Redondo, J. Voces, J.L. Del Estal, J.G. Prieto. Small intestinal sulphoxidation of albendazole. Xenobiotica, 25 (1995), pp. 433–441.
10. Valois, M.E., Takanayagui, O.M., Bonato, P.S., Lanchote, V.L., Carvalho, D., Determination of albendazole metabolites in plasma by HPLC. J. Anal. Toxicol., 1994;18(2), 86-90.
11. Kitzman, D., Cheng, K.J., Fleckenstein, L., HPLC assay for albendazole and metabolites in human plasma for clinical pharmacokinetics studies. Journal of Pharmaceutical and Biochemical Analysis, 2002: 30(3), 801-813.