Available online on 15.06.2024 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research

Open Access

Review Article

Bioanalytical Method Development and Validation of Antihelmintic Drug in Human Plasma

Pandey A. Sapna*, Monika P. Jadhao

Department of Quality Assurance, Vidya Bharti College of Pharmacy Amravati Maharashtra 444602.

A B S T R A C T

Triclabendazole is an anthelmintic or anti-worm medication. Asimple and sensitive bio-analytical HPLC method with UV detection was developed and validated triclabendazole in human plasma. The analytes were extracted from human plasma samples by liquid-liquid extraction technique. Triclabendazole showed maximum absorbance i.e.; λmax at 305 nm. The developed analytical method was validated as per ICH guidelines. The chromatographic separation was achieved with Hypersil BDS C18 (250 mm x 4.6 mm, Particle size: 5 µm), software Autochrom 3000 using a mobile phase composition of acetonitrile and buffer (60:40 V/V) at a flow rate of 1.2 mL/min with a run time of 7 min.

The method showed good linearity in correlation coefficient (r) of >0.9998. The LOD and LOQ were found to be 1.00 µg mL-1 and 3.02 µg mL-1, respectively. Accuracy and precision data were found to be less than 2%, indicating the suitability of method. The developed HPLC method in human plasma using protein precipitation extraction for sample preparation was found to be very simple, reliable, precise, accurate, sensitive and selective analytical method for the estimation of Tricalbendazole. The method is suitable for routine quantitative analysis in pharmaceutical dosage forms. The method developed can be used in therapeutic drug monitoring units, bioequivalence and bioavailability studies, pharmacokinetic and toxicology studies of Tricalbendazole.

Keywords: Bioanalytical Method, Triclabendazole, human plasma,

A R T I C L E I N F O: Received 02 Jan 2024; Review Complete24 March 2024; Accepted 18 May 2024 ; Available online 15 June. 2024

Cite this article as:

Sapna PA, Jadhao MP, Bioanalytical Method Development and Validation of Antihelmintic Drug In Human Plasma,Asian Journal of Pharmaceutical Research and Development. 2024; 12(3):169-175 DOI: http://dx.doi.org/10.22270/ajprd.v12i3.1414

*Address for Correspondence:

Pandey A. Sapna, Department of Quality Assurance, Vidya Bharti College of Pharmacy Amravati Maharashtra444602.

INTRODUCTION

The creation and validation of analytical methods are crucial processes in the discovery, research, and production of pharmaceuticals. Quality control laboratories use the official test methods that emerge from these processes to verify the identity, purity, potency, and performance of drug products. These methods encompass all the procedures that demonstrate a specific technique for quantitative measurement of analytes in a given biological matrix, such as blood, plasma, serum, or urine, and are reproducible, dependable, and appropriate for the intended use.[1]Recent research indicates that sample throughput plays a crucial role in the development of bioanalytical methods that need effective preparation.[2] The analysis that was done in this way needs to be validated and confirmed to serve the intended goal.

It is important to conduct an inquiry at every stage to ascertain if the external milieu, the matrix, or the procedural factors have the potential to impact the analyte estimation in the matrix from the point of collection to the analysis. The development of new procedures has benefited greatly from advancements in analytical instrumentation in recent times.[3,4]

Triclabendazole (Egaten, Novartis Pharmaceuticals, East Hanover, NJ, USA) was authorized by the US Food and Drug Administration (FDA) in February 2019 for the management of human fascioliasis.[5] Through a donation program set up under the direction of the World Health Organization's (WHO) Department of Control of Neglected Tropical Diseases, triclabendazole has been used in many other parts of the world after receiving permission in Egypt in 1997 and France in 2002. In this article, the history of triclabendazole treatment for Areview is conducted of human fascioliasis, focusing on the more recent literature.

One of the most prevalent illnesses in humans is helminthic, a parasitic worm that lives in the intestines of humans and is spread by food, drink, and the air. Helminthic emits toxins, consumes essential nutrients from the host body, and causes disease.[6] Drugs used to cure illnesses in animals caused by

parasitic worms are referred to as antihelmintics. In addition to round worms (nematodes), this also contains flat worms such as flukes (trematodes) and tapeworms (cestodes).

The parasites are of huge importance for human tropical medicine and for veterinary medicine. Present treatment regimen for these diseases have limitations as the currently used anthelmintic drugs are mainly micro fiilaricidal, with little effect on the adult worms; hence new drugs are urgently required. In this regard, natural products have made and continue to make important contributions to this therapeutic area. . Present treatment regimen for these diseases have limitations as the currently used anthelmintic drugs are mainly micro fiilaricidal, with little effect on the adult worms; hence new drugs are urgently required.[7] In this regard, natural products have made and continue to make important contributions to this therapeutic area.

Two species of flukes, Fasciola hepatica and Fasciola gigantica, are the cause of fascioliasis. Their intricate life cycles involve both freshwater snails and mammalian hosts, and they primarily infect goats, cattle, and sheep. However, humans can contract the disease if they ingest larval flukes (metacercariae) in contaminated water or aquatic vegetables.[8] F. hepatica is widespread in Europe, the Americas, Asia, Africa, and Oceania. It can grow up to 30 mm in length. Both species are found in portions of Asia, southern and eastern Africa, while western Africa are home to the bigger (75 mm) F. gigantica.[9,10]

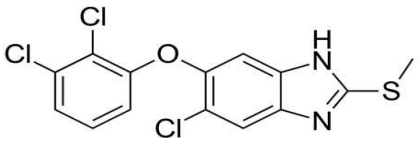
There are 1.1 million in Africa and 1.3 million in Latin America.[11] In endemic areas, school-age children are disproportionately impacted. Fasciolasis can be a dangerous infection in both adults and children, causing neurological aftereffects, an enlarged, painful liver, high fever, and anemia.[12,13]Natural catastrophes, especially flooding, have the potential to spread fascioliasis more widely and cause outbreaks. Though they are uncommon, reported deaths do happen.9. Fascioliasis is linked to serious sickness and morbidity, particularly in lower-class farming areas, and it causes enormous financial losses for the livestock business.

Two stages can be distinguished between typical human fascioliasis patients.[3]The acute stage, which can extend from three to five months, is thought to be connected to fluke migration into the bile ducts. Fever, anorexia, hepatomegaly and splenomegaly, urticaria, abdominal pain, gastrointestinal problems, ascites, anemia, and eosinophilia may accompany it. Although uncommon, respiratory symptoms can also manifest.[10-13]

Ananthelmintic and derivative of benzimidazoles, triclabendazole (tri cla ben' a zole) is used to treat fascioliasis. Two trematode species that infect livestock and can subsequently infect humans are Fasciola hepatica and gigantica. Chemical formula of triclabendazole 5-(methylthio)-2-(3-dichlorophenoxy)-6-(1H-benzimidazole).

The foundation of the majority of effective fasciolosis control strategies is the use of medicines. Triclabendazole (Fasinex®, Novartis) is one of the most widely used drugs for treating fasciolosis worldwide. Since TCBZ is highly effective against both adult and juvenile flukes as young as one week old, it is usually the first choice anthelmintic for treating livestock for F. hepatica.

When treating animals with immature or mature flukes, TCBZ is the most widely used and effective anthelmintic. TCBZ has been shown to be remarkably effective against Fasciola species in numerous tests pertaining to its use.



TRICLABENDAZOLE
Mol. wt: 359.7 G/MOL
Mol. formula: $C_{14}H_9CL_3N_2OS$

Figure 1: Triclobenzazole's chemical formula and structure.

MATERIALS AND METHOD

Active pharmaceutical ingredients of Triclabendazole were obtained from Sun Remedies Pvt. Ltd. Jaipur, Rajasthan. HPLC Grade waterwere provided by the Thermo Fisher Scientific, Indiaacetonitrile, and acetic acid were purchased from Merk life science.

CHROMATOGRAPHIC CONDITIONS

The chromatography pump (model SP930 D) was equipped with a UV-detector (model730D). The analysis was carried out using Hypersil BDS C18 (250mm x 4.6mm, Particle size: 5 µm), software Autochrom 3000 was used for chromatogram generation and data analysis. The HPLC analysis was conducted Ambienttemperature condition. The mobile phase (pH 3.6) consisted solution of Acetonitrile and buffer (60:40v/v). The volume of injection was 20 µL with solvent flow of 1.2 ml/min. Mobile phase, stock and working solutions were sonicated for 5 min to use. The sample

(Triclabendazole) detection was carried out at 305nm.

PREPARATION OF STOCK SOLUTIONS

Accurately weighed quantity 25 mg of Triclabendazole (TCB) was dissolved in diluent and volume was made up to 100 ml mark (100 µg/ml). The stock standard solution was diluted further with diluent to get final concentration of about 10 µg/ml of TCB. For the Preparation of Standard solutionpipette out 2ml of stock solution and transferred to 20 ml volumetric flask, further dilute upto mark dilute with diluent, Shake well and sonicate for 2 min filter the solution. After centrifugation the clear supernatant liquid was collected and a quantity of 100mg/L was injected into the HPLC column and chromatograms were recorded.

EXTRACTION PROTOCOL:

Take about 2ml of blood centrifuge at 500 rpm speed For 12 min. and supernant layer of plasma was separated using micropipetteseparated the plasma.It can be separated from whole blood by the process of centrifugation.

METHOD VALIDATION

Validation of the developed method as carried out as perICH guidelines to determine the specificity, linearity, accuracy, precision, LOD, LOQ. And Ruggedness.

LINEARITY

Plotting the average peak area at each level versus the concentration in µg/ml reveals that the linearity graph is a straight line. This method yielded a typical calibration curve of correlation equation, and it proven to be linear between µg/ml of Tricalbendazole in human plasma.

Table 1: Calibration standards peak area

Con. (5446ppm or ug/ml)	**Area**
12.50	615.8121
18.75	913.4877
25.00	1215.8496
31.25	1551.7928
37.50	1826.9846
Correlation coefficient (r) (NLT 0.995)	0.9997
Intercept	0.525
Slope	48.97

The chromatograms of the plasma calibration standards with concentrations 12.50, 18.75 , 25.00, 31.25 and 37.50 µg/ml were recorded and shown in figures (Fig 2.) and their peak areas of drug was noted. The calibration curve for tricalbendazole was plotted as peak Area vs. concentration of the tricalbendazolecalibration standards in plasma. The correlation coefficient of tricalbendazoleshown was 0.9993 which was within limits. This calibration curve plotted was linear and showed that the method had adequate sensitivity to the concentration (12-37 µg/ml) of the drug. Finally the data obtained, in this was within limits.

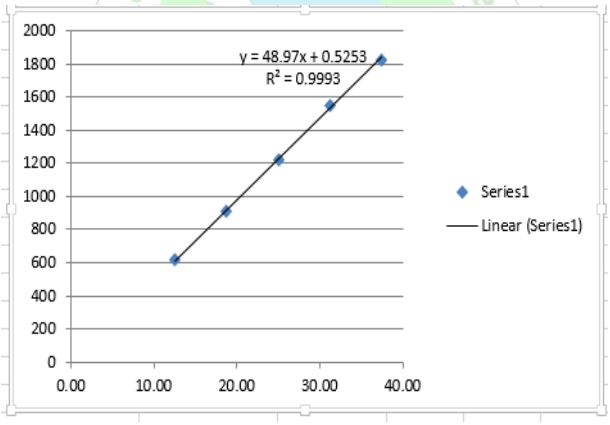


Figure 2: Calibration curve for Triclabendazole

ACCURACY

Acquisition studies were conducted using the standard addition method wherein a known amount of Tricalbendazole was added to the pre-analyzed samples according to 80, 100, and 120% levels of labeled claim further subjected to the contemplated analytical process. The percent recovery and relative standard deviation (% RSD) were calculated for each concentration. Accuracy was calculated using the formula %RE = (E − T) (100/T), where E is the experimentally determined concentration, and T is the theoretical concentration.

Table 2: Accuracy

Accuracy	**Mean % Recovery**	**SD**	**% RSD (NMT)**
Accuracy 80 %	100.58	0.8090	0.80
Accuracy 100 %	99.55	0.9764	0.98
Accuracy 120 %	99.77	1.3577	1.36

PRECISION

Precision was determined by carrying two replicates of concentration and performed intraday (within a day) and interday (day to day) studies. The percentage relative standard deviation (%RSD) was found to be less than 2%. For Intraday precision study, evaluation was carried out by injecting a

standard solution at various time intervals and %RSD of Tricalbendazole was found to be 0.71% shown in table 3 where inter-day precision was carried out in consecutive days with %RSD of 0.96% shown in (table 4). The %RSD can be reached up to 2%. Since the outcome is less than 2% it was found to be satisfactory, which indicates method is precise [8].

Table 3: Intra-Day precision data of Triclabendazole

Name	**Preparation**	% ASSAY
Set-1	prep-01	101.06
	prep-02	99.47
Set-2	prep-01	99.84
	prep-02	99.7
Mean		100.0175
SD		0.7115
% RSD (NMT (NMT 2.0)		**0.71**

Table: 4 Inter-Day precision data of Triclabendazole

Name	**Preparation**	% ASSAY
Day-1	prep-01	101.06
	prep-02	99.47
Day-2	prep-01	101.73
	prep-02	100.40
Mean		100.665
SD		0.9641
% RSD (NMT 2.0)	T 2.0)	**0.96**

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

Limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Whereas the limit of quantitation refers to the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Both are expressed in terms of concentration. The LOD and LOQ were calculated by using slope value of linearity curve. The results are enlisted in Table-5

Table: 5 LOD & LOQ Results

Parameters	**Results**
Slope	48.9704
LOD (µg/ml)	1.00
LOQ(µg/ml)	3.02

ROBUSTNESS

The robustness of the developed method was studied by evaluating the effect of slight but deliberate variations in chromatographic conditions. The parameters studied were flow rate and mobile phase composition.

SYSTEM SUITABILITY

These parameters were shown to be within specified limits. Column efficiency (theoretical plates), resolution factor and peak asymmetry factor, tailing factor, LLOQ are the system suitability parameters. These parameters of the optimized methods were found satisfactory. The results of the system suitability studies in plasma. These parameters were shown to be within specified limits.

Table: 6 System Suitability

Name	**Area**	**RT(min)**	**TP (NLT 2000)**	**TF (NMT 2.0)**

Standard _Inj_01	1195.7273	4.217	10605	1.03
Standard_Inj_02	1187.0009	4.283	14917	0.99
Standard_Inj_03	1186.8041	4.267	10472	1.04
Standard_Inj_04	1183.6812	4.300	11857	0.98
Standard_Inj_05	1222.3322	4.217	10232	1.07
Mean	1195.1091	4.257		
SD	15.8650	0.0382		
%RSD (NMT 2)	**1.33**	**0.90**		

RESULT AND DISCUSSION:

Chromatogram of Tricalbendazole standard

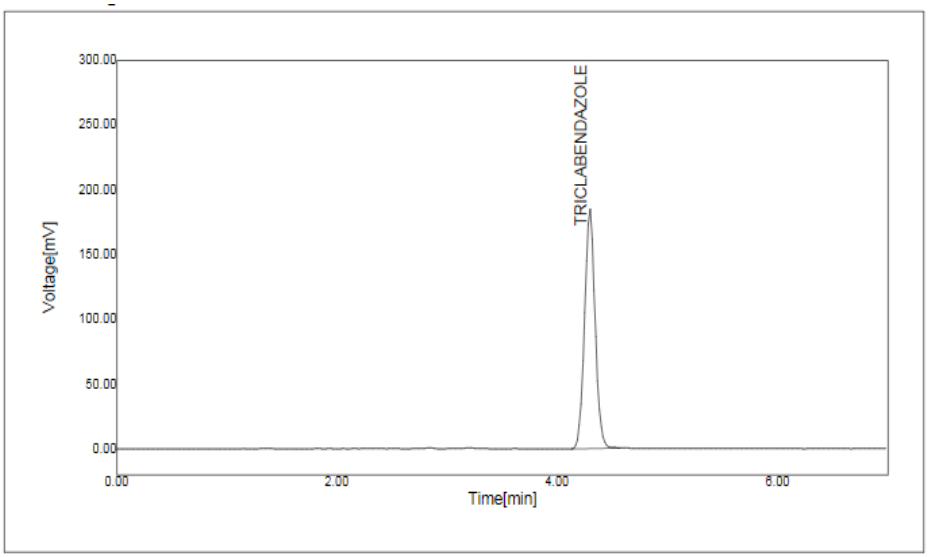


Figure 3: Chromatogram of Tricalbendazole standard

METHOD VALIDATION:-

ACCURACY:

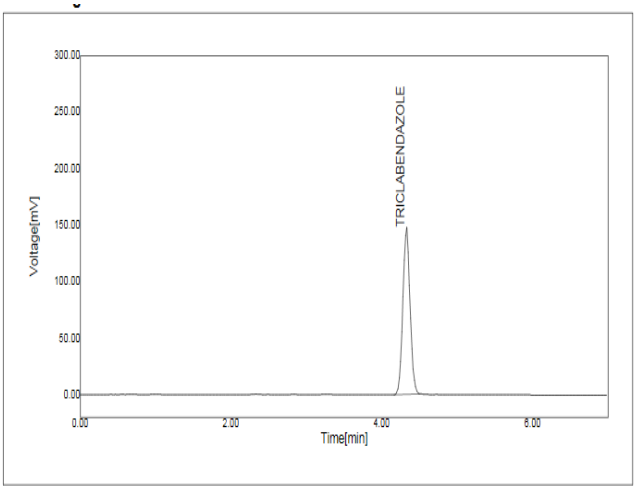


Figure: 4 Chromatogram 80 %

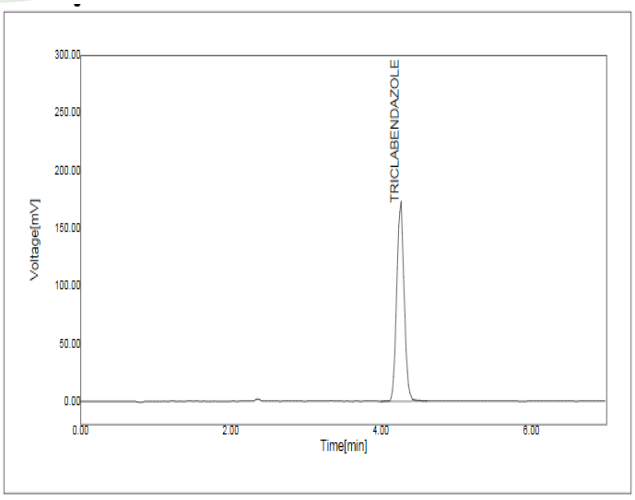


Figure: 5 Chromatogram 100 %

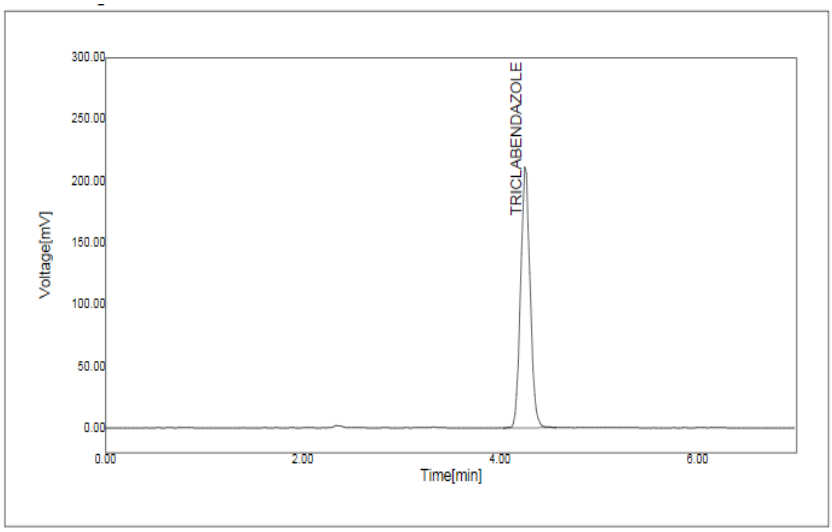


Figure 6: Chromatogram 120 %

CONCLUSION

A Bioanalytical method was developed for the estimation of Triclabendazole in Human Plasma by HPLC method and was validated. Triclabendazole is an anthelmintic drug. The method was developed using Acetonitrile and buffer in the ratio of 60:40. v/v. The peaks obtained for the drug of interest by the present method was symmetrical in nature with acceptable tailing factor and from the plasma endogenous proteins by Protein precipitation Extraction. The retention time of Triclabendazole was shorter and proves that the method is rapid. All the analytical validation parameters were determined and found in the limit as per ICH guidelines, which indicates the validity of the method. The method was validated with respect to accuracy, precision, linearity and robustness. The results of linearity, intraday and interday precision study and capability of the extraction method were within the limits of Bioanalytical method development. The method was linear with a correlation coefficient of acceptable agreement, which is suitable for the estimation of Triclabendazole in human plasma. From the current work it was finally concluded that the developed HPLC method in human plasma using protein precipitation extraction for sample preparation was found to be very simple, reliable, precise, accurate, sensitive and selective analytical method for the estimation of Triclabendazole. The method is suitable for routine quantitative analysis in pharmaceutical dosage forms. The method developed can be used in therapeutic drug monitoring units, bioequivalence and bioavailability studies, pharmacokinetic and toxicology studies of Triclabendazole.

REFERENCES

1. International council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, ICH Guideline M10 on Bioanalytical Method Validation; 2019. p. 5-20.
2. Centr for Drug Evaluation and Research. Bioanalytical Method Validation Guidance for Industry, US Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Bio pharmaceutics. Center for Drug Evaluation and Research; 2018. p. 5-11.
3. Sonawane LV. Bioanalytical method validation and its pharmaceutical application a review. Pharm Anal Acta 2014;5:2153-435.
4. Rajput D. Validation of analytical methods for pharmaceutical analysis. Int J Pharm Erud 2013;3:31-40.
5. US Food and Drug Administration. Drug Trials Snapshots: EGATEN. https://www.fda.gov/drugs/drug-trials-snapshots-egaten [accessed 29 April 2019].
6. K. D. Chatterjee, Parasitology, Protozoology and Helminthology, Guha Ray, Sree Saraswaty Press, Calcutta, India, 1967.
7. N. Mathew, S. Misra-Bhattacharya, V. Perumal, and K. Muthuswamy, "Antifilarial lead molecules isolated from Trachyspermum ammi," Molecules, vol. 13, no. 9, pp. 2156–2168, 2008.
8. Mas-Coma S, Bargue MD, Valero MA. Human fascioliasis infection sources, their diversity, incidence factors, analytical methods and prevention measures. Parasitology. 2018145 (13 Special Issue):1665–1699
9. Mas-ComaS,BarguesMD,ValeroMA.Diagnosis of human fascioliasis by stool and blood techniques: update for the present global scenario. Parasitology. 2014141 (14):1918–1946.
10. Mas-Coma S, Valero MA, Bargues MD. Chapter 2. Fasciola, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Ad Parasitol. 2009; 6941–146.
11. Furst T, Keiser J, Utzinger J. Global burden of human food borne trematodiasis: a systematic review and metaanalysis. Lancet Infect Dis. 201212(3):210–221.Furst T, Keiser J, Utzinger J. Global burden of human food borne trematodiasis: a systematic review and metaanalysis. Lancet Infect Dis. 201212(3):210–221.
12. Lopez M, White AC Jr, Cabada MM. Burden of Fasciola hepatica infection amongchildrenfromPaucartamboinCusco,Peru.AmJTropMed Hyg. 2012;86(3):481–485.
13. Mas-Coma S, Argument VH, Valero MA. Neurological and ocular fascioliasis in humans. Adv Parasitol. 2014;84:27–149.
14. MillánJC,MullR,FreiseSetal.Theefficacyandtolerabilityof triclabendazole in Cuban patients with latent and chronic Fasciola hepatica infection. Am J Trop Med Hy. 2000;63(5–6):264–269.
15. Arjona R, Riancho JA, Aguado JM et al. Fascioliasis in developed countries: a review of classic and aberrant forms of the disease. Medicine (Baltimore). 1995;74(1):13–23.
16. Marcos LA, Terashima A, Gotuzzo E. Update on hepatobiliary flukes: fascioliasis, opisthorchiasis and clonorchiasis. Curr Opin Infect Dis. 200821(5):523–530.
17. Kaya M, Be¸sta¸s R, Cetin S. Clinical presentation and management of Fasciola hepatica infection: single-center experience. World J Gas troenterol. 2011;17(44):4899–4904.
18. Keiser J, Engels D, Büscher G et al. Triclabendazole for the treat ment of fascioliasis and paragonimiasis. Expert Opin Investig Drugs. 2005;14(12):1513–1526.

19. GoralV,SenturkS,MeteOetal.AcaseofbiliaryFascioliasisbyFasciola gigantica in Turkey. Korean J Parasitol. 2011;49(1):65–68.

20. Hawramy TA, Saeed KA, Qaradaghy SH et al. Sporadic incidence of fascioliasis detected during hepatobiliary procedures: a study of 18 patients from Sulaimaniyah governorate. BMC Res Notes. 2012; 5:691

21. MusaD,Godbole G, Chiodini PL et al. Unusual case of a lung abscess. BMJ Case Rep 2013;2013: bcr2012008306. 18 Cabada MM, White AC Jr. New developments in epidemiology, diagnosis, and treatment of fascioliasis. Curr Opin Infect Dis. 2012;25(5):518–522.