Research Article

HPLC method for simultaneous determination of Albendazole metabolites in plasma

Z. Khalil[1], M. El karbane[2], M. Azougagh[2], J. El harti[1] and J. Taoufik[1]

[1]Laboratory of Medicinal Chemistry, Faculty of Medicine and Pharmacy, University Mohammed V, Rabat, Morocco
[2]Physicochemical Service, Drugs Quality Control Laboratory, Division of Drugs and Pharmacy, Ministry of Health, Rabat, Morocco

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ABSTRACT

A simple, sensitive and reliable high performance liquid chromatography (HPLC) method has been developed and validated to simultaneous determination of albendazole metabolites: albendazole sulphoxide (ABZSO) and albendazole sulphone (ABZSO$_2$) in plasma. The chromatographic separations were achieved on a reversed phase C_{18} column (250x4.6 mm, 5µm) using a mixture of acetonitrile/ammonium phosphate buffer (0.025M, pH=5), the flow a rate UV detection were set at 1.2 ml/min and 295 nm, respectively. The total run time for the method was within 18 min. The method was found to show a good linearity in the concentration range of 0.01 - 2 µg/ml for both analytes (ABZSO and ABZSO$_2$) with a correlation coefficient of 0.998 and 0.999 for ABZSO and ABZSO$_2$, respectively. The assay was accurate and reproducible with a quantification limit of 0.01µg/ml for ABZSO and ABZSO$_2$. Intra-day coefficients of variation of ABZSO and ABZSO$_2$ were 3.75 and 3.39 % respectively, while the recovery accuracy was between 81.39% for ABZSO and 81.66% for ABZSO$_2$. This method appears to be robust and has been successfully applied to a pharmacokinetic study of albendazole in dogs.

Keywords: Albendazole, Analysis, Plasma, HPLC, Validation.

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INTRODUCTION

Benzimidazoles (BZD) are anthelmintic drugs widely used in veterinary and human medicine, they have generally broad spectrum of activity and low toxicity.

Albendazole, methyl [5-(propylthio)-1H-benzimidazol-2-yl] carbamate, is an anthelmintic benzimidazole with an excellent activity for the treatment of intestinal helminthiasis and echinococcosis [1]. It's an effective drug for prevention and treatment of these diseases but the therapeutic response in echinococcosis is unpredictable due to poor bioavailability [2, 3].

Clinical studies have reported poor absorption of albendazole, with wide interindividual variability in the plasma concentrations as a consequence of its low solubility, thus high doses and long treatment are required to achieve therapeutic effects [4, 5]. After oral administration, albendazole (ABZ) undergoes first-pass metabolism by liver microsomal enzymes [6, 7] where it is quickly metabolized in albendazole sulphoxide (ABZSO), pharmacologically its major active metabolite ; Subsequently, this metabolite is further transformed by cytochrome P450 enzyme (CYP2C) to an inactive metabolite, albendazole sulphone (ABZSO$_2$) [8, 9]. Due to this extensive metabolism, the parent compound is undetectable after administration in blood plasma, in various animal species and humans, [10, 11],for this reason many pharmacokinetic studies are developed to plasma concentrations of ABZSO and ABZSO$_2$ [12, 13]. Thus, the anthelmintic effect observed after oral administration of albendazole can be attributed to the presence and activity of ABZSO, whereas detection and quantification of ABZSO$_2$ provide additional information about the overall metabolism.

To date, literature offers published methods for the determination of ABZ metabolites by high performance liquid chromatography (HPLC) with UV or fluorescence detection [14].

Most of the albendazole metabolite analysis in body fluids with UV detection methods were unable to determine efficiently the albendazole metabolites simultaneously, and use a gradient for separation, which prolongs the analysis run times [15, 16] .

Mirfazaelian et al.[17] have described an HPLC assay for all three albendazole metabolites in human serum. However, the method involved both, a UV (ABZSO) and a fluorescence detector (ABZSO2 and ABZSO2NH2), displayed poor chromatographic characteristics, and provided relatively high limits of quantification, along with laborious sample pretreatment.

Furthermore, Bogan and Marriner reported an HPLC assay of albendazole in plasma and gastrointestinal (GI) fluids [18]. The procedure involved liquid–liquid extraction and had a recovery of 80%, with a sensitivity of 0.02 µg/ml but using of large volumes of samples plasma (4ml).

In this context, the aim of the present study was to develop and validate a simple, sensitive, reproducible and robuste high-performance liquid chromatography (HPLC) method with UV detection (using isocratic elution with one step liquid–liquid extraction and small volume of samples plasma) for the simultaneous determination of albendazole metabolites (ABZSO, ABZSO$_2$) in plasma, which will useful for application for a pharmacokinetic study.
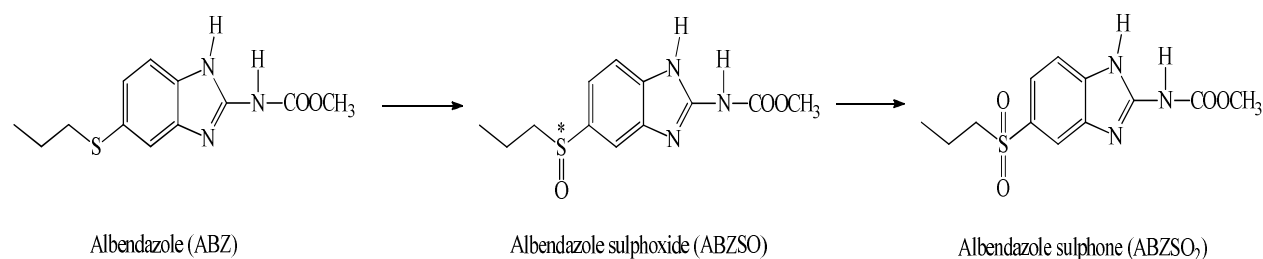

Fig.1. Chemical structures of Albendazole (ABZ), albendazole sulphoxide (ABZSO) and albendazole sulphone (ABZSO$_2$)

EXPERIMENTAL SECTION

Reagents and chemicals
Albendazole was obtained from Mercure Pharmaceutical Ltd, Vadodara (India) and mebendazole from Toronto Research Chemicals Inc., (Toronto, Canada) was used as an internal standard (IS) for the analytical method validation. ABZSO and ABZSO$_2$ (purity > 97 %) were synthesized in the laboratory of Medicinal Chemistry, Faculty of medicine and Pharmacy - Rabat.

Ethyl acetate (purity 99.5%) was from Solvachim and all others chemicals were purchased from Sigma Aldrish. The blood plasma was supplied by the « National Centre for Blood Transfusion – Rabat – Morocco », and stored frozen in aliquots at - 20°C.

Standard stock solutions
Stock solutions were made with ABZSO and ABZSO$_2$, containing 100µg/ml and were dissolved in methanol in volumetric flasks and the solution was sonicated for 15 min. Separately mebendazole (MEB) was also prepared at concentration of 500µg/ml.

Working standard solutions
The working solutions were prepared in methanol to final concentrations of 20, 5, 1, 0.5, 0.1 µg/ml for each analyte. A 50µg/ml solution of mebendazole was prepared as working solution for the internal standard, the solutions were stored at - 4°C.

Chromatographic conditions
Experimental and plasma samples were analyzed for ABZSO, ABZSO$_2$, by high performance liquid chromatography (HPLC Waters 2695, USA). HPLC analysis was carried out using a ultraviolet detector (Photodiode Array detector 2996). The metabolites was monitored by a wavelength of 295 nm. The chromatographic separation was carried out on a C$_{18}$ reversed phase Thermohypersil ODS (4.6 × 250 mm, 5 µm) column that was thermostated at 25 ∘C.

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The mobile phase consisted of acetonitrile/ammonium phosphate buffer (0,025M, pH=5), Elution from the stationary phase was carried out at a flow rate of 1.2 ml/min in isocratic mode and the total run time was set to 18 min. Data were processed using the Empower Pro 2 data integrator., The method was validated according to ICH Q2(R1) guidelines [19].

Extraction procedure

Plasma samples of 800µl were transferred in a 15ml glass tubes, and spiked with 100µl of mebendazole solution (internal standard, 50 µg/ml), after vortex mixing 100µl of working solution (20, 5, 1, 0.5, 0.1µg/ml) of ABZSO, $ABZSO_2$ were added.

Extraction of ABZSO, $ABZSO_2$, and MEB was preformed by a liquid-liquid extraction by addition 3.6 ml of ethyl acetate as extraction solvent. Subsequently, the tubes were capped, shaken horizontally for 20 min and then centrifuged for 10 min at 2000g to allow phase separation,

The organic phases were transferred to clean tubes and the solvent was evaporated to dryness in a water bath at 55 ∘C under a stream of nitrogen. The residues were dissolved in 1 ml mobile phase, after vortex mixing, 50µl of sample subsequently injected for chromatographic system.

Method validation:

The developed method was validated by evaluating specificity, linearity, precision, accuracy and quantification limit.

Specificity: The specificity of the method was determined by comparing the chromatograms of drug free plasma samples with those spiked with standard solutions.

Linearity: The linearity of the method was evaluated using calibration samples of five concentrations for each analyte by triplicate analysis (n=3) for each level. Calibration curves in ranges 0.01-0.05-0.1- 0.5 to 2µg/ml for ABZSO and $ABZSO_2$ have been constructed. The data were analyzed for linearity using the least-squares regression and evaluated by its coefficient of determination (r^2).

Precision: The evaluation of the intra-day precision was determined by evaluation of replicates (n = 6) of drug-free plasma samples fortified with each compound (ABZSO, $ABZSO_2$) at concentration of 0.1 µg/ml within a single day. Inter-day variation was determined by 6 replicates analysis (n = 18) of drug-free plasma samples spiked with ABZSO, and $ABZSO_2$ at concentration of 0.1 µg/ml during three consecutive days. The precision was expressed as coefficient of variation (CV %).

Accuracy:

Accuracy of the analytical method was calculated by the determination of five replicates of blank samples fortified at three different spiked levels (0.05- 0.1 and 0.5µg/ml) with ABZSO and ABZSO2 over a period of 3 days.

The absolute recoveries of each compound (ABZSO, ABZSO2) from plasma were determined by comparing the peak areas obtained from fortified blank samples with the peak areas resulting from direct injections of equivalent quantities of standard solutions.

Detection and quantification limits:

The quantification limit of the assay was defined at the lowest detected concentration on the standard curve that would result in coefficient of variation less than 20%. Limit of detection (LOD) was estimated by LOD = 3.3σ/S where σ is the residual standard deviation of the regression line, S is the slope of the standard curve.

RESULTS AND DISCUSSION

Separation and samples pretreatment

The new method was validated, with respect to linearity, specificity, accuracy, precision, detection and quantification limits.

Fig.1 shows the chemicals structures of the molecules assayed in the current work.

Preliminary experiments involving volumes of extraction solvent was performed for optimisation of extraction efficiency. The procedure employed for the extraction of ABZSO and $ABZSO_2$ from plasma samples was simple and efficient in removing endogenous interferents.

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Thus, A good baseline separation was achieved for all albendazole metabolites, no interferences peaks were found at the retention times of ABZSO and ABZSO$_2$ and MEB.

Fig.2 shows the chromatograms of samples spiked with , ABZSO, ABZSO$_2$ and MEB (IS).

All were eluted within 18 min with the respective retention times of ABZSO, ABZSO$_2$, and MEB were approximately 3.57 , 5.93 and 17.31 min, respectively.
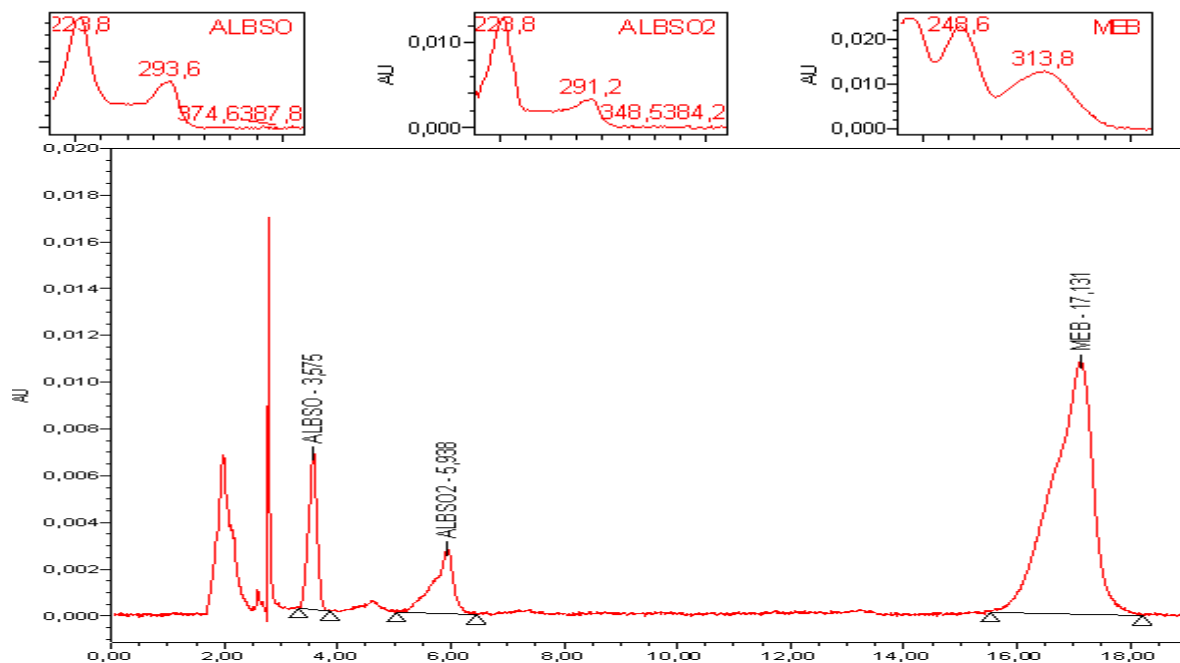

Fig.2. Chromatographic separation of albendazole sulphoxide (ABZSO),albendazole sulphone (ABZSO2) and mebendazole (MEB) used as inetrnal standard

Linearity

The calibration curves for ABZ metabolites in plasma were obtained using the linear least-squares regression procedure. A good linearity was demonstrated over the calibration range for the assay (0.01, 0.05, 0.1, 0.5, 2 µg/ml for ABZSO, and ABZSO$_2$).

Regression analysis of the data provided the following equations to describe the calibration curves: y= 0.248 x + 0.002, for ABZSO, y= 0.210 x + 0.0003 for ABZSO$_2$, where y represents the peak area and x is the quantity (in µg/ml) of each compound. Correlation coefficients (r²) of the calibration curves ranged between 0.998 and 0.999 for ABZSO and the ABZSO$_2$, respectively.

Table 1: Validation data of analytical method for determination of ABZSO and ABZSO$_2$ from spiked plasma samples

Parameters	ABZSO	ABZSO$_2$
Linearity :		
Range (µg/ml) :	0.01 – 2	0.01 – 2
Determination coefficient (r²)	0.998	0.999
Quantification limit (µg/ml) :	0.01	0.01
Detection limit (µg/ml) :	0.003	0.003
Intra-day precision (CV %), n=6	3,75	3,39
Inter-day precision (CV %), n=18	4,94	5,73
Accuracy :		
0.05 µg/ml	81.96 %	84.13 %
0.1 µg/ml	83.14 %	81.59 %
0.5 µg/ml	80.09 %	80.25 %
Range of recovery:	78.25 – 85.34 %	78.62 – 86.01 %

Precision and Accuracy:

The chromatographic analyses at different concentrations values of ABZSO and ABZSO$_2$ were used repeatedly to determine intra- and inter-day precision and accuracy. The data results are presented in Table 1. The intra-day precision values varied from CVs: 3.75 and 3.39% for ABZSO and ABZSO$_2$, respectively, and the inter-day precision showed optimal values with CVs between 4.94 and 5.73% for ABZSO and ABZSO$_2$ respectively.

The mean estimated recoveries were 81.39% and 81.66% for ABZSO, ABZSO$_2$, respectively.

The results demonstrate that the extraction efficiency is relatively constant over the range considered and the accuracy varied from 78.25 to 86.01 % for both metabolites.

These results indicate an acceptable accuracy and precision (<15%) values for the albendazole metabolites determination on the same or different days.

Detection and quantification limits
The lower concentrations in the calibration curves were considered as a quantitation limit of the method 0.01 µg/ml for both ABZSO and ABZSO$_2$.

The detection limit was estimated as 0.003µg/ml for both analytes (ABZSO and ABZSO$_2$), respectively. The values were low and accurate enough for the application of the method to pharmacokinetic studies.

Application of the method
This method was successfully applied to pharmacokinetic study of five dogs who received a single oral dose of albendazole (10mg/kg). Serial samples were collected up to 24h after the last medication dose. A plasma concentration profile obtained in the analysis is presented Fig 3.
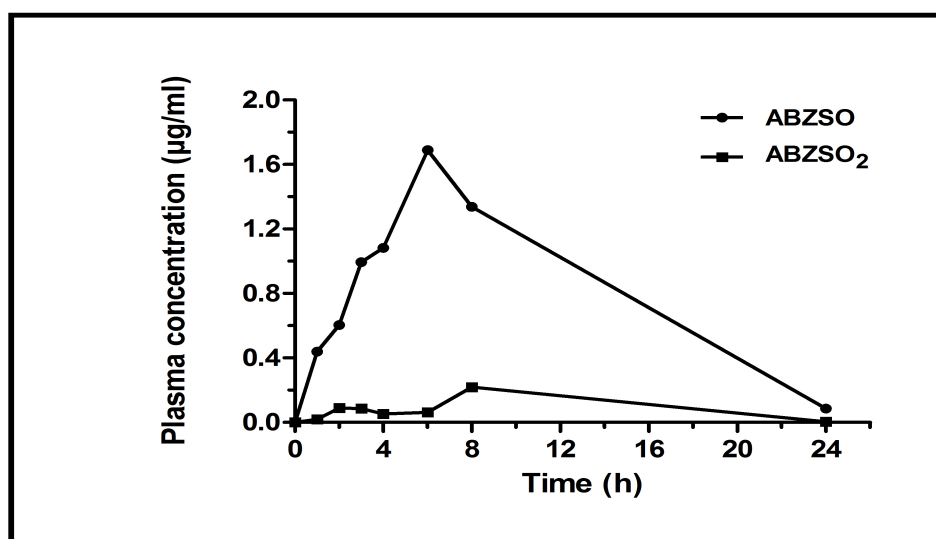

Fig.3. A plasma concentration-time profile for a dogs who received an oral dose of albendazole

Albendazole is poorly absorbed from the gastrointestinal tract owing to its low water solubility being rapidly converted to the ABZSO producing the systemic anthelmintic activity [20]. For this reason, the detection of the main drug (ABZ) is not important from in vivo analytical view point, confirming the findings of earlier studies [21, 22].

The chromatographic system described here is suitable for the separation and determination of ABZ metabolites in plasma samples within 18 min in. In our method, the LOQ of 0.01µg/ml for ABZSO and ABZSO$_2$ is more sensitive and selective than others reported in the literature using HPLC coupled to UV detection [15-16, 23-24].

In addition, the high recovery about 81%, was similar to those obtained by Gokbulut et al. with liquid-liquid extraction [24].

After oral administration, albendazole was not detected during the acquisition time, while the metabolites (ABZSO and ABZSO$_2$) appeared relatively rapidly in dogs plasma, which confirmed the early results [25]. These results confirm that albendazole is extensively metabolized in dogs.

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CONCLUSION

In summary, the analytical method described in this study was developed and validated for simultaneous quantification of ABZ metabolites in plasma. This method was precise, robust and simple for application in pharmacokinetic studies and clinical practice, in which large numbers of samples are usually analysed.

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