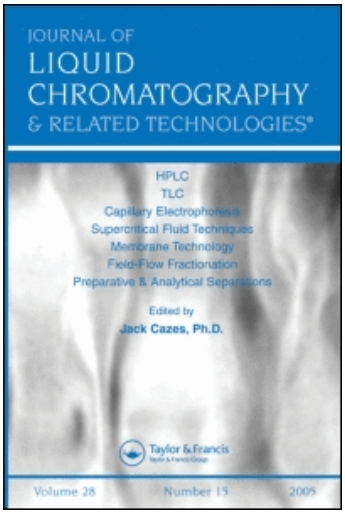

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The Determination of Albendazole by Flow Injection Analysis Method Using UV-Detection and HPLC Method in Suspensions

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Abstract: A simple flow injection analysis (FIA) method of albendazole (ALB) using UV detection and a HPLC method are described in this study. For the FIA method, the best solvent system was found to consist of methanol : HCl : water (49 : 1 : 50). The limit of detection (LOD) and limit of quantitation (LOQ) were calculated to be 1.2×10^{-8} and $3.7 \times 10^{-8}\,\mathrm{mol \cdot L^{-1}}$ (n = 8), respectively. For the HPLC method, the mobile phase is a mixture of methanol:acetate buffer (0.05 M, pH:5.8) (70 : 30 v/v). The retention time obtained was 8.07 min. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated to be 1.1×10^{-8} and $3.8 \times 10^{-8}\,\mathrm{mol \cdot L^{-1}}$ (n = 8), respectively. The proposed methods were applied to the determination of ALB in the suspensions. The results were compared with those obtained from UV spectrophotometry. The validation studies were realised by the related applications and the results were evaluated statistically.

Keywords: Albendazole, Flow injection analysis, High performance liquid chromatography, Pharmaceutical analysis

INTRODUCTION

Albendazole, 5-(propylthio)-1H-benzimidazol-2-yl-carbamic acid methyl ester, (ALB) is an anthelmintic drug, and it is active against most of the nematode and some cestode worms in human and animals[1,2] (Figure 1).

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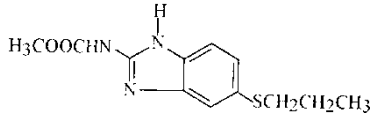

Figure 1. The chemical structure of ALB.

Several studies have been reported for the determination of ALB, such as HPLC,[3–9] GC-MS,[10] UV spectrophotometr,[11] non aqueous capillary electrophoresis,[12] adsorptive stripping voltammetry,[13] and differential pulse voltammetry.[14,15]

Flow injection analysis (FIA) is a new methodology characterised by its versatility, ease of automation, high sampling frequency, and minimum sample treatment prior to injection into the system. The FIA techniques have found wide applications recently, mainly due to reduction of the analysis time and reagents consumption compared to conventional manual procedures. On the other hand, their high sensitivity makes them suitable for the determination of low concentrations of pharmaceuticals in biological fluids when used as detectors in HPLC. They can also optimise the detection of analyte independently from the way the process occurs in the chromatographic column.[16] A special apparatus is used in the method of FIA. But such a complex apparatus is not used for the direct determinations. Only a peristaltic pump is sufficient. Thus, the analyte can be transmitted into the detector directly by using an HPLC pump. This application simplifies the FIA method.

The aim of this study was to investigate and develop direct determination of ALB by FIA by a simple, rapid, precise, and accurate reversed-phase HPLC method, using UV detection, as an applicaton for pharmaceutical dosage forms. In addition, the results were compared with those obtained from UV spectrophotometry.

EXPERIMENTAL

Apparatus and Chemicals

For the FIA method, the HPLC apparatus used was a Model LC 6A pump equipped with a 20 μL manual loop injector, a Model SPD-A10 UV variable wavelength detector, and a Model C-R7A integrator (all Shimadzu, Japan). For chromatography, the HPLC system consisted of a Model Spectra System SCM 1000 degasser, Spectra System P1000 isocratic pump, Spectra System SN4000 connector, Spectra System UV6000LP diode array detector (Thermo Finnigan, USA). The analyte peaks were resolved at the ambient temperature on a Phenomenex Nucleosil C18 (150 × 4.6 mm I.D.; particle size 5 μm) column. The volume of injection loop was 20 μL. The data were collected and analysed with Chrom Quest™ 4.0 HPLC database

system on a IBM Pentium IV computer. A Shimadzu Spectrophotometer Model UV 2401 PC (Japan) and quartz cells for the measurement of the absorbance for UV spectrophotometry were used.

Standard ALB (99.8%) and its pharmaceutical preparation, Andazol® suspension, containing $20\,mg \cdot mL^{-1}$ active material were kindly supplied from Biofarma A.S. (Istanbul, Turkey). Other chemicals were of analytical grade of Merck (Germany).

Solutions

A stock solution of ALB ($1 \times 10^{-3}\,mol \cdot L^{-1}$) was prepared using methanol : HCl : water (49 : 1 : 50). The dilutions were made with the same solvent. As a carrier phase an aqueous solutions of MeOH (10%, v/v) was used for the FIA method. For the HPLC method, stock solutions of ALB ($1 \times 10^{-3}\,mol \cdot L^{-1}$) was prepared in methanol : HCl : water (49 : 1 : 50). Standard solutions of ALB were prepared with the mobile phase in the range of 2.0×10^{-7} to $3.0 \times 10^{-6}\,mol \cdot L^{-1}$. Twenty microliters of each solution was injected into the column and chromatograms were recorded.

Chromatographic Conditions

The proposed HPLC procedure was conducted using the reversed-phase technique. The mobile phase is a mixture of methanol : acetate buffer ($0.05\,mol \cdot L^{-1}$, pH : 5.8) (70 : 30). The mobile phase was prepared daily, filtered, and sonicated before use. The mobile was filtered through 0.45 μm membrane filter and degassed for 20 min before use and pumped from the reservoir to the column at the rate of $1\,mL \cdot min^{-1}$. The absorbances at 230 nm were recorded.

Spectrophotometric Studies

The stock solution of $1 \times 10^{-3}\,mol \cdot L^{-1}$ from standard ALB in methanol : HCl : water (49 : 1 : 50), and a series of standard solution in the concentration range 1×10^{-5}–$5 \times 10^{-5}\,mol \cdot L^{-1}$ diluted from the stock solution, were prepared. The spectrophotometric measurements were made at 230 nm using quartz cuvets.

Analysis of Pharmaceutical Dosage Forms

For the pharmaceutical analysis, the suspension containing $20\,mg \cdot mL^{-1}$ was homogenated in an ultrasonic bath for 15 minutes. Then, 0.5 mL of the suspension was transferred into a 100 mL flask and dissolved in methanolic HCl. A half

portion of the solution was centrifuged at 3000 rpm for 10 minutes. The clear solution was filtered through a 0.45 μm membrane filter. The supernatant was diluted to predetermined values and injected into a sample loop by means of a syringe.

RESULTS AND DISCUSSION

An ALB solution of $3.0 \times 10^{-6}\,\text{mol} \cdot \text{L}^{-1}$ was used to determine the parameters for the optimisation and a solvent system consisted of methanolic HCl. The optimum concentration of MeOH, in view of peak morphology, was determined to be 10% (v/v). Since the flow rate of the carrier is an important parameter affecting the sensitivity of the method, it was gradually changed from $0.2\,\text{mL} \cdot \text{min}^{-1}$ to $2\,\text{mL} \cdot \text{min}^{-1}$ and the best flow rate was found to be $1.3\,\text{mL} \cdot \text{min}^{-1}$. The area under the curve values of ALB in methanolic HCl were measured at 230 nm. All experiments were performed at ambient temperature. When the base line was reached, another sample was injected. The signals of the ALB at concentrations ranging from 2.5×10^{-7} to $3.0 \times 10^{-6}\,\text{mol} \cdot \text{L}^{-1}$ were obtained under the given conditions (Figure 2).

For the HPLC analysis of ALB, a reversed-phase isocratic procedure is proposed as a suitable method. A mixture of methanol : acetate buffer (0.05 M, pH : 5.8) (70 : 30 v/v), at a flow rate of $1\,\text{mL} \cdot \text{min}^{-1}$ was found to be an appropriate mobile phase, allowing adequate and rapid separation of ALB. A typical chromatogram for the analysis of ALB is shown in Figure 3. Under these conditions, the retention times of 8.07 ± 0.05 min for the ALB were obtained. The retention times of ALB were highly precise. The calibration curve for ALB was calculated by plotting of the peak normalisation against drug concentrations. The linear range of ALB was found to be

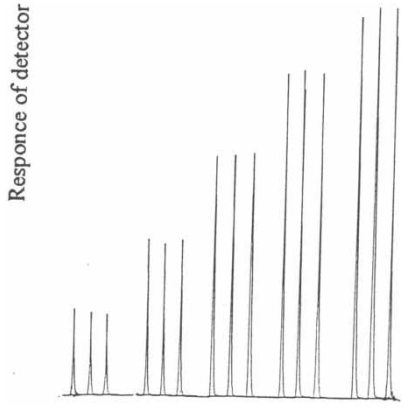

Figure 2. The signals in the 2.5×10^{-7}–$3 \times 10^{-6}\,\text{mol} \cdot \text{L}^{-1}$ concentration range of ALB.

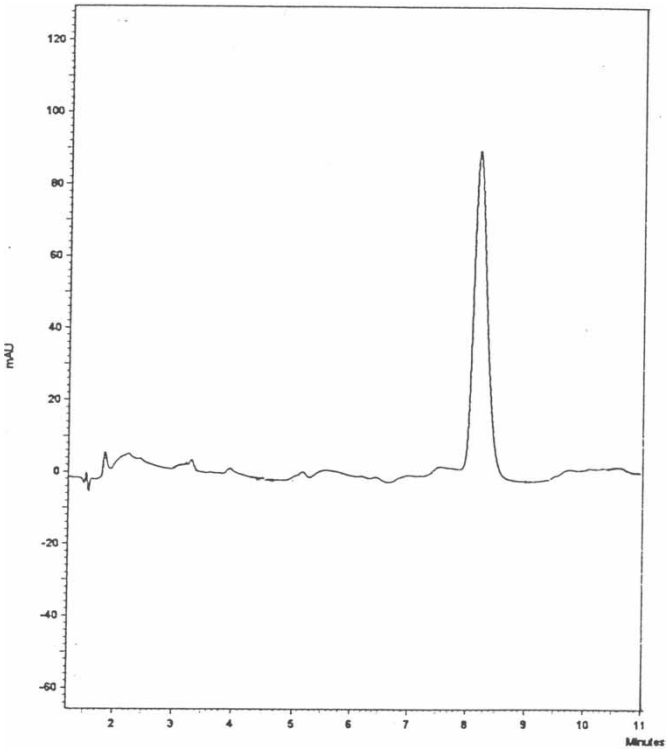

Figure 3. A typical chromatogram of ALB ($1 \times 10^{-6}\,\mathrm{mol \cdot L^{-1}}$).

3×10^{-7}–$1 \times 10^{-6}\,\mathrm{mol \cdot L^{-1}}$. The characteristics of regression equations for both methods are given Table 1.

The methods were validated for its intra- and inter-day precision. As seen in Table 2, in the range of 2×10^{-7}–$1 \times 10^{-6}\,\mathrm{mol \cdot L^{-1}}$, RSD values were

Table 1. The regression parameters of FIA and HPLC methods

Parameter	FIA	HPLC
Linearity range($\mathrm{mol \cdot L^{-1}}$)	2.5×10^{-7}–3.0×10^{-6}	3×10^{-7}–$1. \times 10^{-6}$
Slope	1.904×10^{10}	216183.2
Intercept	26348	0.0027
Correlation coefficient	0.9997	0.9998
RSD of slope	1.87	1.18
RSD of intercept	0.46	0.35
LOD ($\mathrm{mol \cdot L^{-1}}$)	1.2×10^{-8}	1.1×10^{-8}
LOQ ($\mathrm{mol \cdot l^{-1}}$)	3.7×10^{-8}	3.8×10^{-8}

Table 2. Intra- and inter-day precision for the determination of ALB

	Observed concentration of ALB							
	FIA				HPLC			
Concentration of ALB (10^{-7} mol · L^{-1})	Intra-day		Inter-day[a]		Intra-day		Inter-day[a]	
	Mean[b]	RSD	Mean[b]	RSD	Mean[b]	RSD	Mean[b]	RSD
2	2.08	0.25	2.04	0.36	2.11	0.33	2.01	0.26
4	4.12	0.38	4.10	0.41	4.20	0.41	4.08	0.50
6	6.11	0.36	6.07	0.58	6.13	0.56	5.98	0.78
8	8.24	0.42	8.13	0.63	8.17	0.48	8.16	0.51
10	10.25	0.48	10.17	0.70	10.14	0.60	10.10	0.65

[a]Each value is the average of five experiments.
[b]On three different days.

obtained between 0.25 and 0.70. These data indicate a considerable degree of precision and reproducibility for the proposed methods, both during one day and between different days. Sample solutions, analyzed after one week, did not show any appreciable change in assay values. No interfering peaks were found in the chromatogram, indicating that the suspension excipients did not interfere. Known amounts of the analyte at 6, 8, and 10 μg concentration levels were added and assayed for the recovery of ALB from the standard solution and suspensions. The results obtained are given in Table 3. The lower RSD values of the assay indicate that the proposed methods are highly precise and accurate.

Table 3. Recovery and accuracy for the determination of ALB

	Recovery from standard solution		Recovery from suspension	
Amount Added (μg)	Mean[a] ± RSD amount (μg)	Mean[a] ± RSD recovery	Mean[a] ± RSD amount (μg)	Mean[a] ± RSD recovery
FIA				
6	6.14 ± 0.16	102.3 ± 0.18	5.94 ± 0.19	99.0 ± 0.47
8	8.20 ± 0.23	102.5 ± 0.44	7.91 ± 0.52	99.9 ± 0.41
10	9.98 ± 0.56	99.8 ± 0.85	10.03 ± 0.72	100.3 ± 0.52
HPLC				
6	6.01 ± 0.20	100.2 ± 0.26	5.91 ± 0.28	98.5 ± 0.36
8	8.05 ± 0.12	100.6 ± 0.35	8.11 ± 0.41	101.4 ± 0.43
10	10.24 ± 0.69	102.4 ± 0.87	10.14 ± 0.71	101.4 ± 1.12

[a]Each value is the average of five experiments.

Table 4. Assay results of ALB as percent in suspensions

	FIA	HPLC	UV-spectrophotometric
Mean	99.3	101.2	99.8
n	8	8	8
RSD%	1.12	1.16	0.96
Confidence limit (p = 0.05)	±1.84	±1.18	±1.53
t- test of significance	1.38	1.67	$t_{0.05} = 2.14$ (table)
F-test of significance	2.46	3.17	$F_{0.05} = 4.17$ (table)

[a]Andazol® suspensions containing 20 mg · ml^{-1} ALB.

There is generally no need for a comparison method for the validated method. But in addition to these studies, the UV spectrophotometric studies for ALB has also been achieved for support of these results. Calibration studies were done by preparing standard solutions in the range of 1×10^{-5}–5×10^{-5} mol · L^{-1} concentration. The relationship between absorbance (A) and concentration of ALB (C) was found to be $A = 22857.6\,C\ (mol \cdot L^{-1}) + 1.18 \times 10^{-3}$; $r = 0.9998$. at 230 nm. The determination methods in the study were applied to the suspensions, and the results tabulated in Table 4. The results were also evaluated statistically. No statistical significant difference was observed between the methods at the 95% probability level (F- and t-test). The results of the study indicated that the proposed methods are suitable, simple, precise, rapid, and accurate for the determination of ALB in pharmaceutical dosage forms.

REFERENCES

1. Schulz, M.; Peruche, B. Albendazole. A new anthelminthic. Pharm. Ztg. **1994**, *139*, 39–41.
2. Li, T.; Qiao, G.L.; Hu, G.Z.; Meng, F.D.; Qiu, Y.S.; Zhang, Y.Y.; Guo, W.X.; Yie, H.L.; Li, S.F.; Li, S.Y. Comparative plasma and tissue pharmacokinetics and drug residue profiles of different chemotherapeutics in fowls and rabbits. J. Vet. Pharmacol. Ther. **1995**, *18*, 260–273.
3. VanTonder, E.C.; De Villiers, M.M.; Handford, J.S.; Malan, C.E.P.; Du Preez, J.L. Simple, robust, and accurate high-permormance liquid chromatography method for the analysis of several anthelmintics in veterinary formulations. J. Chromatogr. A **1996**, *729*, 267–272.
4. Fletouris, D.; Botsoglou, N.; Psomas, I.; Mantis, A. Rapid quantitative screening assay of trace benzimidazole residues in milk by liquid chromatography. J. AOAC Int. **1996**, *79*, 1281–1287.
5. Fletouris, D.; Botsoglou, N.A.; Psomas, I.E.; Mantis, A.I. Trace analysis of albendazole and its sulfoxide and sulfone metabolites in milk by liquid chromatography. J. Chromatogr. B., Biomed. Appl. **1996**, *687*, 427–435.
6. Chu, P.S.; Wang, R.Y.; Brandt, T.A.; Weerasingle, C.A. Determination of albendazole-2-aminosulfone in bovine milk using high performance liquid chromatography. J. Chromatogr. B., Biomed. Appl. **1993**, *620*, 129–135.

7. Valois, M.E.C.; Takayanagui, O.M.; Bonato, P.S.; Lanchote, V.C.; Carvalho, D. Determination of albendazole metabolites in plasma by HPLC. J. Anal. Toxicol. **1994**, *18*, 86–90.
8. Botsoglou, N.A.; Fletouris, D.J.; Psomas, I.E. Ion-pair liguid chromatographic determination of albendazole in cattle feed. J. Liq. Chromatogr. & Rel. Technol. **1999**, *22*, 2297–2305.
9. Fregonezi-Nery, M.M.; Baracat, M.M.; Kedor-Hackmann, E.R.M.; Pinheiro, R.M. Determination of albendazole in oral suspension. Anal. Lett. **2001**, *34*, 1255–1263.
10. Markus, J.; Sherma, J. Method II. Gas chromatographic / mass spectrometric confirmatory method for albendazole residues in cattle liver. J. AOAC Int. **1992**, *75*, 1135–1137.
11. Mandal, S.C.; Bhattacharryya, M.; Maity, A.K.; Gupta, B.K.; Ghosal, S.K. Determination of albendazole in tablet formulation by UV- spectrophotometric method. Indian Drugs **1992**, *29*, 323–324.
12. Prochazkova, A.; Chouki, M.; Theurillat, R.; Thormann, W. Therapeutic drug monitoring of albendazole: Determination of albendazole sulfone in human plasma using non-aqueous capillary electrophoresis. Electrophoresis **2000**, *21*, 729–736.
13. Abu-Zuhri, A.Z.; Hussein, A.I.; Musmar, M.; Yaish, S. Adsorptive stripping voltammetric determination of albendazole at a hanging mercury drop electrode. Anal. Lett. **1999**, *32*, 2965–2975.
14. Atkosar, Z. The differential pulse voltammetric determination of albendazole in suspensions using rotating glassy carbon electrode. Acta Pharm. Turc. **2001**, *33*, 43–46.
15. DeOliveria, M.F.; Stradiotto, N.R. Voltammetric assay of albendazole in pharmaceutical dosage forms. Anal. Lett. **2001**, *34*, 377–387.
16. Gonzales, F.; Tarin, P.; Maspoch, S.; Blanco, M. Flow injection amperometric determination of pharmaceutical. Arch. Pharm. **1988**, *321*, 725–728.

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