Received: 15 November 2022 | Revised: 17 January 2023 | Accepted: 18 January 2023

DOI: 10.1002/elps.202200273

ELECTROPHORESIS

SHORT COMMUNICATION

An easy and green assay to determine albendazole and ivermectin in veterinary preparations by micellar liquid chromatography

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Funding information
Ministerio de Ciencia e Innovación, Grant/Award Number: PID2019-106708GB-I00MCIN/AEI/10.13039/501100011033; Department of Science and Technology, Ministry of Science and Technology, India, Grant/Award Number: FISTandPURSE-II[grantnumberSl.No.64Dated31-05-2016]; University Grants Commission, Grant/Award Number: JuniorResearchFellowshiptoR.P.P.

Abstract

A procedure to determine albendazole and ivermectin in veterinary formulations, like tablet, bolus, oral suspensions, and injections by micellar liquid chromatography, has been developed. Sample preparation was a batch solid-to-liquid extraction in mobile phase, consisting of a stirring step (15 min), followed by ultrasonication (15 min) and filtration of the obtained supernatant, to reach a target concentration of 2 mg/L for both analytes. Using a mobile phase of 0.15 M sodium dodecyl sulfate—6% 1-pentanol buffered at pH 3 with a 0.01 M phosphate salt, running at 1 mL/min through a C18 column, both drugs were resolved in less than 10 min. Absorbance detection wavelength was 292 nm. Procedure was validated by the guidelines of the International Council on Harmonization in terms of specificity, calibration range (0.025–5 mg/L), trueness (97.8%–102.6%), precision (<2.2%), and system suitability. The method was found easy-to-handle, low cost, safe, green, and with high sample-throughput, thus useful for routine analysis. Therefore, it represents a valuable alternative for quality control of veterinary formulations. It was applied to samples of veterinary formulations purchased from local chemists and veterinarians, and label claims were inside the acceptance criteria (95%–105%).

Abbreviations: ALB, albendazole; FDA, Food and Drug Administration; IVE, ivermectin; *k*, retention factor; MLC, micellar liquid chromatography; *N*, number of theoretical plates; ORA, Office of Regulatory Affairs; PA, peak area; r^2, coefficient of determination; Rs, resolution; RSD, relative standard deviation; *T*, tailing factor.

KEYWORDS
anthelminthic, micellar, suspension, tablet, validation

Ruminants and poultry raised in farms with food purposes are exposed to be colonized by worm-parasites, especially in countries from tropical and subtropical areas [1]. This has a negative impact on feed intake, health disorders, weight gain, mortality rates, meat weight and quality, fertility, milk yield, and animal welfare, what results in a loss of production and economic profit. Therefore, the control of worm-infections is essential to provide enough meat and dairy products to an increasing world population and to ameliorate the economy of the areas living on livestock production [2]. The routine administration of broad-spectrum anthelmintic drugs has been for three decades the most common strategy to prevent and treat worm infections [3].

Albendazole (ALB), a benzimidazole, and ivermectin (IVE), a macrocyclic lactone, are well-tolerated broad-spectrum veterinary anthelminthic agents [4, 5]. Therapies based on ALB + IVE enhance clinical effects compared to ALB or IVE alone [6]. Both compounds, either alone or combined, are commonly used by Indian farmers to control a broad spectrum of parasitic infections in cattle and poultry raised to elaborate meat, milk, and milk-derived products [5]. ALB and IVE are taken orally and marketed under several veterinary formulations, such as tablets, bolus, and oral suspensions. Additionally, IVE can be parentally administered and is distributed as injection [7].

Micellar liquid chromatography (MLC), with sodium dodecyl sulfate-based hybrid micellar solutions as extraction solvent and mobile phases, has been largely used to determine drugs in diverse sorts of dosage forms [8, 9]. Hybrid micellar solutions are made up of mainly safe and eco-friendly chemicals, and a maximum of 15% v/v of organic solvent, which makes an excellent choice in Green Analytical Chemistry, displays excellent solubilization power [10]. An MLC-method to determine ALB and IVE in food and biological waste has been recently developed [5].

The aim of the research was to adapt the method described in Ref. [5] to determine ALB and IVE in several kinds of veterinary preparations (tablets, bolus, oral suspension, and injections) used in bovine and poultry farms. The procedure should be validated to prove its suitability. The method was applied to dosage forms obtained from several chemists and veterinaries from Sagar area (Madhya Pradesh, India).

Reagents, standards, preparation of solutions, apparatus, instrumentation, chromatographic conditions, and the calculations of the chromatographic parameters are described in Ref. [5]. Various veterinary products available in the form of tablets, bolus, injectable, or oral suspension containing ALB and IVE in combination or alone were analyzed (Table 1). The samples were randomly taken from several chemists and veterinary clinics from Sagar and Chhindwara city in the state of Madhya Pradesh, India. Ten tablets or bolus from the same package were crushed into fine powder with an Agatha mortar, whereas oral suspension and injections were kept in their original package. All the veterinary samples were kept in the fridge at +4°C until analysis. They were processed the same day of the analysis, and the treated samples were discarded after analysis, as they remain stable until 2 days after being prepared [5]. An amount of 2.5 g sample and 20 mL mobile phase were placed in an Erlenmeyer flask and shaken using a magnetic stirrer for 15 min. Thereafter, the mixture was ultrasonicated for 15 min, filtered, and transferred to a 25-mL-volumetric flask, which was filled up to the mark. Finally, an aliquot was diluted to get a target concentration of 2 mg/L. Although the sample preparation takes a long time, the stirring and ultrasonication steps are performed without hand manipulation, as the operator only has to do the mixture and introduce it in the apparatus. Therefore, it remains easy-to-conduct.

Sample treatment was optimized by adapting the procedure described in Ref. [5], based on a batch stirring-assisted solid-to-liquid extraction followed by a batch ultrasound-assisted solid-to-liquid extraction. The studied parameters and tested values were solvent (mobile phase, methanol, and pure micellar solution of 0.15 M SDS), pH (3, 5, 7, and 9), and time of stirring and time of ultrasonication (5 + 5, 10 + 10, 15 + 15, and 20 + 20 min, respectively). These were optimized for each sort of sample and anthelmintic, by comparing the relative recovery following a one-factor-a-time approach. The formulations selected for the study were (see Table 1) Albeder IR (ALB and IVE in tablet), Bandykind Plus (ALB and IVE in bolus), Hitek Injection (IVE in injections), and Hymin Plus (ALB and IVE in oral suspensions). The sample-to-solvent ratio was set to 1:10. Relative recoveries inside 97%–103% were considered acceptable [11].

Relative recoveries were roughly similar for ALB and IVE. Otherwise, the results for liquid samples were slightly higher than for solid ones. Extraction using mobile phase (98.2%–100.1%) and methanol (98.5%–100.2%) provided similar results, and then the first one was preferred to

TABLE 1 Description of the studied veterinary preparations based on albendazole (ALB) and/or ivermectin (IVE) and their respective label claim

Veterinary preparation	Trade name	Manufacturer	Amount of active principle	Label claim (%)
Tablet	A1	Ceza Formulations Pvt Ltd (Indore, Madhya Pradesh, India)	ALB 400 mg	102.3
Tablet	Iverfast	Sharvik Impex India Pvt Ltd (Nagpur, Maharashtra, India)	IVE 6 mg	97.0
Tablet	Abide	Laborate Pharmaceutical India (Delhi, India)	ALB 400 mg	104.3
Tablet	ABL	Malar Healthcare (Ahmedabad, Gujarat, India)	ALB 400 mg	94.0
Bolus	Livealth Bolus	Livealth Biopharma Private Limited (Mumbai, India)	ALB 300 mg	99.7
Injection	Hitek Injection	Virbac India (Mumbai, India)	IVE 10 mg in 1 mL	99.0
Oral suspension	Albomar	Virbac India	ALB 25 mg in 90 mL	98.0
Bolus	Crebenex	Credence Remedies Pvt. Ltd (Thane, Maharashtra, India)	ALB 300 mg	103.1
Bolus	Feulimec-10	Sriwalls Healthcare	IVE 10 mg	98.7
Tablet	Albeder IR	Wonder Healthcare (Delhi, India)	ALB 400 mg + IVE 12 mg	98.4/101.3
Oral Suspension	Bandy Plus	Mankind Pharma Limited (Delhi, India)	ALB 200 mg + IVE 12 mg in 5 mL	95.4/96.34.0
Oral suspension	Hymin Plus	Cross Berry Pharma (Pilani, Rajasthan, India)	ALB 200 mg + IVE 1.5 mg in 5 mL	100.5/96.7
Bolus	Bandykind Plus	Mankind Pharma Limited	ALB 3 g + IVE 100 mg	98.9/95.3

reduce the volume of organic solvent. The pure micellar solution offered lower relative recovery in all cases (77.6%–86.6%) and then was discarded.

The pH barely influences the relative recovery (97.5%–100.5%), and then the same pH of the mobile phase was chosen (3). For liquid samples, similar relative recovery was obtained, regardless of the extraction time (97.9%–100.6%). However, for solid samples, 5 + 5 min was not enough to extract all the amount of the anthelmintic drugs (<97%), whereas longer times provided adequate results (97.4%–99.9%). The selected experimental time was 15 + 15 min to guarantee the maximal quantity of ALB and IVE passes to the solvent (98.5%–99.6%).

The method was validated following the guidelines of the International Council of Harmonization [12] and the ORA (Office of Regulatory Affairs) FDA (Food and Drug Administration) Methods, Method Verification and Validation [11]. The validation was separately performed for each drug, except for specificity, which was examined simultaneously for the two compounds in the same chromatographic run.

Specificity was evaluated for each anthelmintic drug and kind of veterinary preparation by a peak purity study [8]. The dosage forms taken for the study were those employed in the sample treatment optimization. A standard solution containing 2 mg/L of ALB and IVE was analyzed (Figure 1), and the UV absorbance spectra were recorded between 220 and 400 nm at five points: the maximum height, and at

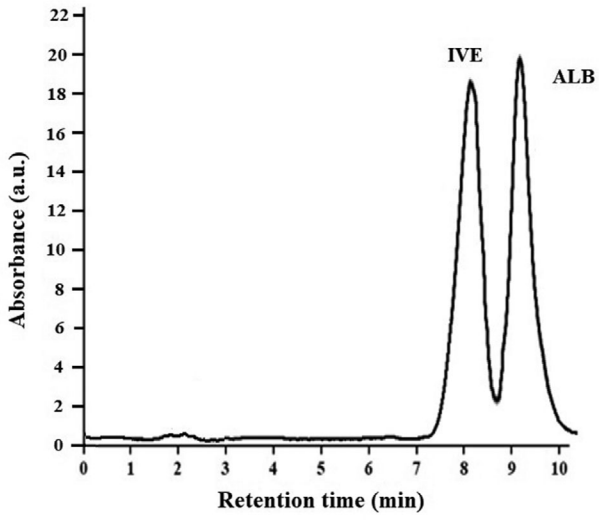

FIGURE 1 Chromatogram obtained by the analysis of a standard solution of 2 mg/L albendazole (ALB) and ivermectin (IVE).

50%- and 5%-peak height at the leading and tailing edge. Furthermore, a sample was treated to get a supernatant containing 2 mg/L of the studied drug and analyzed. Retention times obtained in standard solutions and veterinary preparations were similar (difference <2.0%) The shape of the analyte peak and the UV absorbance spectrum taken for each point, from the chromatogram obtained from the

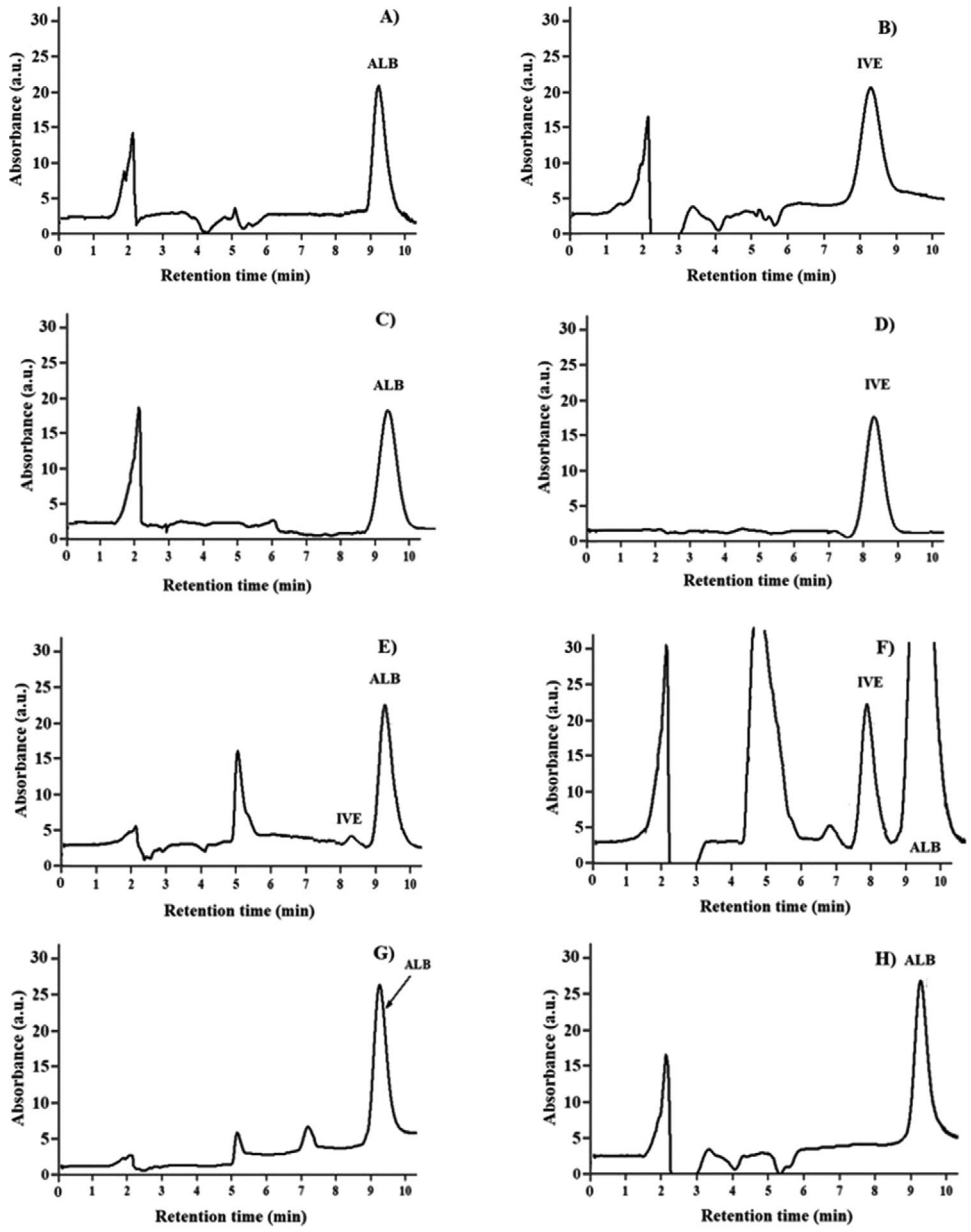

FIGURE 2 Chromatograms obtained by the analysis of veterinary preparations: (A) A1, (B) Iverfast, (C) Abide, (D) Hitek Injection, (E) Bandy Plus (focusing on albendazole [ALB]), (F) Bandy Plus (focusing on ivermectin [IVE]), (G) Livealth Bolus, and (H) Albomar.

analysis of the standard and that of each sort of sample and anthelminthic drug, were compared by overlaying and were found quite comparable in all cases. Otherwise, neither irregularities nor shoulders were noticed in any of the chromatograms. Therefore, there is not any overlapping with excipients or impurities, and then the entire peak area (PA) can be considered from ALB or IVE.

Calibration range and linearity were studied by the analysis of nine standard solutions of increasing concentrations of ALB and IVE up to 5 mg/L by sextuplicate. For both analytes, the model was homoscedastic throughout the entire concentration range by an *F*-test at a significance level of 5%. Least-square linear regression was used to adjust a first-grade equation relating the PA (response) and the concentration (independent variable). The constructed equations were as follows:

$$\text{IVE}: \text{PA} = (8.53 \pm 0.05)\ [\text{IVE}\,(\text{mg/L})] + (0.06 \pm 0.03)\ r^2 = 0.9994$$

$$\text{ALB}: \text{PA} = (7.162 \pm 0.06)\ [\text{ALB}\,(\text{mg/L})] + (0.03 \pm 0.03)\ r^2 = 0.9991$$

The goodness-of-fit of the model was evaluated and found satisfactory by the following tests: Residuals were normally distributed around 0, and no trend was noticed using a Wald–Wolfowitz run test (significance level 5%), no outliers were included in the model as the relative residuals were <3 and the Cook squared distance was <1, the *y*-intercept accounts for less than 1.5% of the target concentration, and its confidence interval includes 0, and there is not a systematic error in the chromatographic analysis, determination coefficients were>0.995, and the relative residual standard deviations were <1.5%. The linear range was 0.025–5 mg/L and then covers 80%–120% of the target concentration (2 mg/L).

The limit of detection and limit of quantification were calculated as 3.3 and 10 times the standard deviation of the blank (estimated as the standard deviation of the *y*-intercept), divided by the slope of the calibration curve. Results were 0.01 and 0.025 mg/L, respectively, for both anthelmintic drugs. The target concentration is largely above LOQ to avoid sensitivity troubles during analysis of veterinary formulations.

Trueness and precision were separately investigated in standard solution (prepared separately from the calibration), at three levels (80%, 100%, and 120% of the target concentration), in intra- and interday conditions. Six standard solutions were consecutively injected. Intraday trueness was the quotient between the average found concentration and the nominal concentration (relative recovery), whereas the interday precision (repeatability) was the relative standard deviation (RSD) of the found concentrations. This approach was repeated for 5 days during a 3-month period, using renewed calibration solutions and samples. Interday trueness was the quotient between the grand mean of the found concentration and the true values, and the interday precision (intermediate precision) was the RSD of all the calculated found concentration (30 values). Closeness of agreement between the average found concentration and the nominal value (97.8%–103.6%), the intraday (<1.9%) and interday (<2.4%) dispersion comply with the acceptance criteria (97%–103%, <2% and <3%) [11]. Therefore, the found concentration determined from a single measurement can be considered trustworthy; hence, no replicate analyses are required.

The consistency and the quality of the chromatographic responses were appraised by a system suitability testing [13]. A standard solution containing 2 mg/L of ALB and IVE was injected six times. The results were (ALB, IVE, and acceptance criteria) as follows: retention time (9.22 ± 0.08; 8.15 ± 0.07 min; etc.), RSD of retention time (0.9;0.9; <1%), RSD of PA (0.9; 0.9; <1.0%), retention factor (5.2;4.4; >2.0); efficiency (2154; 2086; >2000 number of theoretical plates), tailing factor (1.2; 1.4; <2.0), and resolution between both compounds (1.2; >2.0). The obtained chromatographic parameters were adequate to achieve the determination of the analytes, apart from resolution. However, the peaks barely overlap, and their mutual interference is negligible.

Carryover effect, robustness, and stability were already studied in Ref. [5].

The procedure was applied to several veterinary forms, such as tablets, bolus, oral suspensions, and injections containing single (ALB or IVE) or combined (ALB + IVE) dosage forms. The problem associated with simultaneous determination of ALB and IVE in dosage forms is the ratio of both compounds in terms of large difference in quantities. Therefore, the veterinary preparation was analyzed twice, one focusing on ALB, and the other focusing on IVE. Results are in Table 1 and the chromatograms obtained by the analysis of some of them can be seen in Figure 2. All the studied veterinary forms contain the claimed amount of active component (acceptance criteria 95%–105%).

Using this procedure, a single solution must be elaborated, which contains mainly innocuous chemicals, and a low amount of toxic, flammable, and volatile organic solvent (6%). In addition, the reagents, instrumentation, and material required are generic and not expensive. Therefore, the method can be considered green and sustainable.

ACKNOWLEDGMENTS

Work supported by Grant PID2019-106708GB-I00 funded by MCIN (Ministry of Science and Innovation of Spain)/AEI/10.13039/501100011033 and by Department of Science & Technology (Ministry of Science and Technology, Government of India) FIST and PURSE-II [grant number Sl.No.64 Dated 31-05-2016]. We would like to thank University Grants Commission (Government of India) for providing Junior Research Fellowship to R.P.P.

Open access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Pawar R-P, Sharma G, Durgbanshi A, Bose D, Peris-Vicente J, Roca-Genovés P, et al. An easy and green assay to determine albendazole and ivermectin in veterinary preparations by micellar liquid chromatography. Electrophoresis. 2023;1–6. https://doi.org/10.1002/elps.202200273