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Short communication

Rapid determination of the antiparasitic drugs flubendazole and febantel in feeds by HPLC with ultraviolet detection

Guglielmo Dusi*, Valentina Gamba, Elena Faggionato

Department of Chemistry, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna "B.Ubertini", Via Bianchi 9, 25124 Brescia, Italy

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Abstract

A rapid and very effective analytical procedure for the simultaneous HPLC determination of two anthelmintics, Flubendazole (FLUB) and Febantel (FEBA), in swine and poultry feeds was developed and tested.

The ground feed samples were extracted using dimethylformamide. The extracts were directly analyzed, without any purification, on a reversed-phase ODS column (150 mm × 4.6 mm, 5 μm) with acetonitrile–phosphate buffer (pH 3; 0.017 M) as mobile phase. Ultraviolet detection of FLUB and FEBA was carried out at 300 nm.

The method was validated for specificity, linearity, accuracy, repeatability, limit of detection and limit of quantification. The limits of detection (LOD) of FLUB and FEBA in feeds, based on a detector signal-to-noise ratio of 3, were 3 mg kg^{-1} and the lowest levels tested in feeds by this procedure (limit of quantification, LOQ) were 10 mg kg^{-1}.

The mean recovery of FLUB and FEBA from spiked samples, in a concentration range of 10–200 mg kg^{-1}, was 98% with a CV% of 4% and 99% with a CV% of 2%, respectively.

Keywords: Antiparasitic drugs; Anthelmintics; Flubendazole; Febantel; Feeds; Reversed-phase chromatography

1. Introduction

Febantel (FEBA) and flubendazole (FLUB) are anthelmintic drugs belonging to benzimidazoles group. Their wide range of antiparasitic activity qualified them as broad-spectrum drugs with additionally larvicidal and ovicidal properties. Moreover, they have high degree of efficacy, good margin of safety and versatility of administration. They are used both in monogastric and in ruminant animals. Their mode of action acts on parasites by interfering with their energy-generating metabolism without adverse reaction for the host. The way of administration is the oral one, as paste, oral suspension or medicated feed. These drugs are more effective at low-level medication for several days than single dosing and therefore the oral administration, particularly by feeds, is the most useful way in swine- and poultry-breeding. The use of the oral administration for these two benzimidazoles is confirmed by receiving, in our laboratory experience, feeds containing FLUB and FEBA separately. The addition of the drugs to poultry and swine feeds is usually at a range concentration of 30–150 mg kg^{-1} [1,2].

But the production of medicated feeds may lead to some problems, for instance the cross-contamination between medicated and unmedicated feeds and also the production of medicated feeds with lower or higher dosages than the declared ones. For these reasons it is important to control animal feeds in order to provide both qualitative and quantitative information on the drugs, the former to indicate the presence of the antiparasitic products, the latter to indicate whether they are present at the required concentrations. Because of the above-mentioned reasons it is quite clear the importance of a regular control of finished feeds for FEBA and FLUB.

* Corresponding author. Tel.: +39 030 2290341; fax: +39 030 2290562.
E-mail address: gdusi@bs.izs.it (G. Dusi).

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Several analytical methods have been published about the determination of benzimidazoles but literature lacks in articles about feed matrix. Methods published are concerning the determination of several benzimidazoles, alone or in group by HPLC with diode array or mass spectrometer detection, as residues in animal tissues (meat and fish) or foodstuffs (e.g. eggs and milk) [3–8].

To our knowledge there are few articles concerning the analysis of benzimidazoles in feeds [9,10] and none is dealing with the determination of FLUB and FEBA.

In routine analysis one of the main purpose is to have simple methods that can be applied, with the same performances, for multi-drugs determination and for the analysis of different types of matrices or feeds.

The aim of the current work was therefore to develop a rapid and simple analytical method for the simultaneous determination of two benzimidazoles, FEBA and FLUB, in poultry and swine feeds. The method presented is able to detect the analytes at their normal added concentrations as well as at subtherapeutic levels, as a result of a possible cross-contamination at the point of manufacture. Further studies in our laboratory will be done to verify the applicability of the presented method to other benzimidazoles.

2. Experimental

2.1. Reagents

Acetonitrile, HPLC grade, was obtained from BDH (Poole, UK). Orthophosphoric acid 85% was purchased from Carlo Erba (Milan, Italy). Diethylamine was obtained from Fluka Chemie GmbH (Milan, Italy). Dimethylformamide was purchased from Baker-Schilling (Milan, Italy). Water for HPLC analysis was prepared with a Barnstead Nanopure Ultrapure water system from International PBI (Milan, Italy).

2.2. Standard and standard solutions

FLUB and FEBA were generously donated by Istituto Superiore di Sanità (Rome, Italy).

The stock standard solutions of each anthelmintic were prepared in dimethylformamide at the concentration of 1 mg ml^{-1}.

The standard curve solutions, containing both anthelmintics, were prepared by diluting the stock standard solutions to: 1–2.5–5–10–25 μg ml^{-1} in a mixture of water-acetonitrile (50:50, v/v). All the solutions were kept at 4 °C in dark test-tubes.

2.3. Instrumentations

The HPLC system consisted of a HP 1100 Series quaternary pump, a HP 1100 Series diode array detection (DAD) system, a HP 1100 Series autosampler all controlled by a Vectra VE Serie 8 computer using HP Chemstation software (all from Agilent, USA).

2.4. Chromatographic conditions

The HPLC separation was performed at room temperature on a 5 μm Supelcosil LC-18 DB (150 mm × 4.6 mm, 5 μm) column equipped with a Supelguard LC-18 DB (20 mm × 4.6 mm, 5 μm) guard column (both columns from Supelco, Bellefonte, PA, USA).

HPLC eluent A was pure acetonitrile; HPLC eluent B was 0.017 M phosphate buffer (pH = 3 with diethylamine).

The gradient initiated with 30% eluent A for 5 min, continued with a linear increase to 70% eluent A over 12 min and 70% eluent A for 4 min. The system returned to 30% eluent A in 1 min and was re-equilibrated for 4 min before the next injection. The flow rate was 1 ml/min and the injection volume was 10 μl. The wavelength was set to 300 nm.

2.5. Spiked samples

For the recovery studies feed samples spiked with FLUB and FEBA, over a concentration range of 10–200 mg kg^{-1}, were prepared on the day of use by spiking aliquots of blank control feed with the stock standard solutions.

Samples were processed as described in next section.

2.6. Sample preparation and analysis

A representative portion of the ground feed sample was pulverized using a domestic grinder to obtain a homogeneous powder. Ten grams of this powder was weighed into a 250 ml glass jar and extracted with 100 ml of dimethylformamide. The mixture was shaken with a horizontal shaker for 45 min and put in an ultrasonic bath for 15 min at room temperature. About 10 ml of supernatant was centrifuged for 5 min at 3000 rpm. Ten microliters of centrifuged supernatant were injected into the HPLC-DAD system operating as described above. The presence of FLUB or FEBA in the sample were confirmed using the retention time of the chromatographic peaks and also by a co-chromatography.

At the same time with HPLC samples analyses, FLUB and FEBA calibration curves were done in the concentration range 10–250 ng injected (10–250 mg anthelmintics per kg of feed according to this method). These calibration curves were used to quantify the benzimidazoles using the external standard method.

3. Results and discussion

The analytical method developed is based on a HPLC determination of FLUB and FEBA deriving from a crude feed extract without any purification step. The procedure presented was validated for specificity, linearity, accuracy, repeatability, limit of detection and limit of quantification. About specificity Figs. 1a and 2a show the chromatograms of representative blank swine and poultry feed extracts, respectively. As shown some interfering peaks were observed

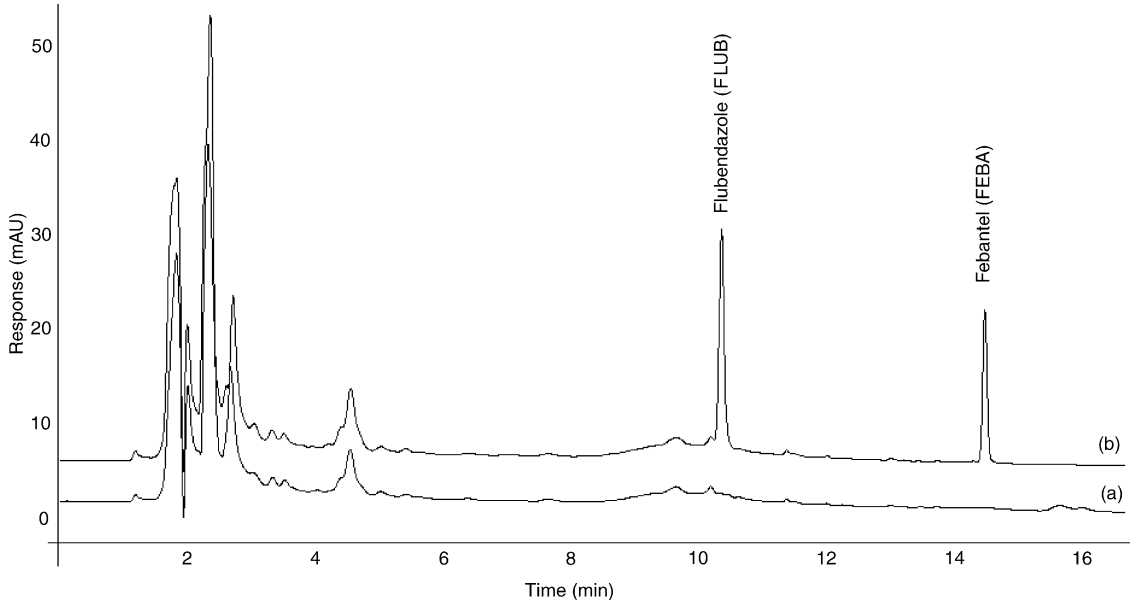

Fig. 1. HPLC chromatograms of swine feed extracts: (a) blank; (b) blank spiked with both FLUB and FEBA at 50 mg kg^{-1}.

on the chromatograms but none of these were present at the retention times of FLUB and FEBA.

The linearity of the photometric detector response to FLUB and FEBA, at 300 nm, was verified, in the range 1–25 μg ml^{-1}, by injecting 10 μl of the standard curve solutions. All the responses, in the concentration range 10–250 ng injected (10–250 mg anthelmintics per kg of feed according to this method) were found to be linear with a correlation coefficient of 0.999 or greater.

The analysis of the chromatograms of six different swine and poultry feed extracts allowed to estimate the LOD.

The described method permits to achieve a LOD, based on a detector signal-to-noise ratio of 3 (S/N = 3), of 3 mg kg^{-1} for both anthelmintics.

The lowest level tested (limit of quantification, LOQ) in fortified feed samples was 10 mg kg^{-1}. Figs. 1b and 2b show the chromatograms of swine and poultry blank feeds (the same as in Figs. 1a and 2a) spiked with FLUB and FEBA at a concentration of 50 mg kg^{-1}, respectively.

To evaluate the accuracy and the repeatability of the method, blank poultry feed samples were spiked in a

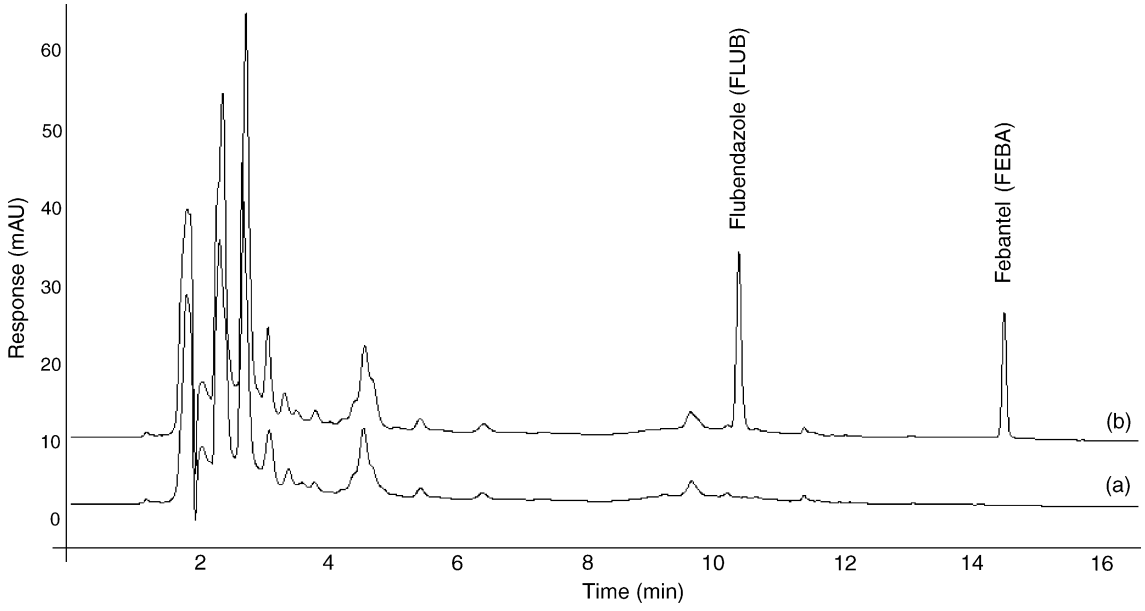

Fig. 2. HPLC chromatograms of poultry feed extracts: (a) blank; (b) blank spiked with both FLUB and FEBA at 50 mg kg^{-1}.

Table 1
FLUB recovery from replicates ($n = 9$) of spiked samples of blank control poultry and swine feeds

FLUB added (mg kg^{-1})	Poultry		Swine	
	Mean recovery (%)	R.S.D. (%)	Mean recovery (%)	R.S.D. (%)
10	99	6	100	7
50	98	2	98	2
100	97	1	96	1
200	96	1	97	1

Table 2
FEBA recovery from replicates ($n = 9$) of spiked samples of blank control poultry and swine feeds

FEBA added (mg kg^{-1})	Poultry		Swine	
	Mean recovery (%)	R.S.D. (%)	Mean recovery (%)	R.S.D. (%)
10	100	4	100	2
50	100	2	100	2
100	99	1	98	1
200	98	1	98	1

Table 3
Assay of commercial swine feed with a declared FLUB concentration of 30 mg kg^{-1}

Replicates	Concentration found (mg kg^{-1})	Mean	R.S.D. (%)
A	26.9	27.8	1.8
B	27.9		
C	27.9		
D	28.4		
E	27.9		
F	28.0		

concentration range of 10–200 mg kg^{-1} for FLUB and FEBA and processed, under repeatability conditions, as described in Section 2.6.

Both anthelmintics were quantified using the external standard method and the results obtained are summarized in Tables 1 and 2 for FLUB and FEBA, respectively.

The mean accuracy (recovery) for FLUB and FEBA from spiked feed samples were 98% and 99%, respectively, while the mean repeatability (R.S.D.) for FLUB and FEBA from spiked feed samples were 3% and 2%, respectively.

The method proposed was tested on a commercial swine feed submitted to our laboratory. The results are given in

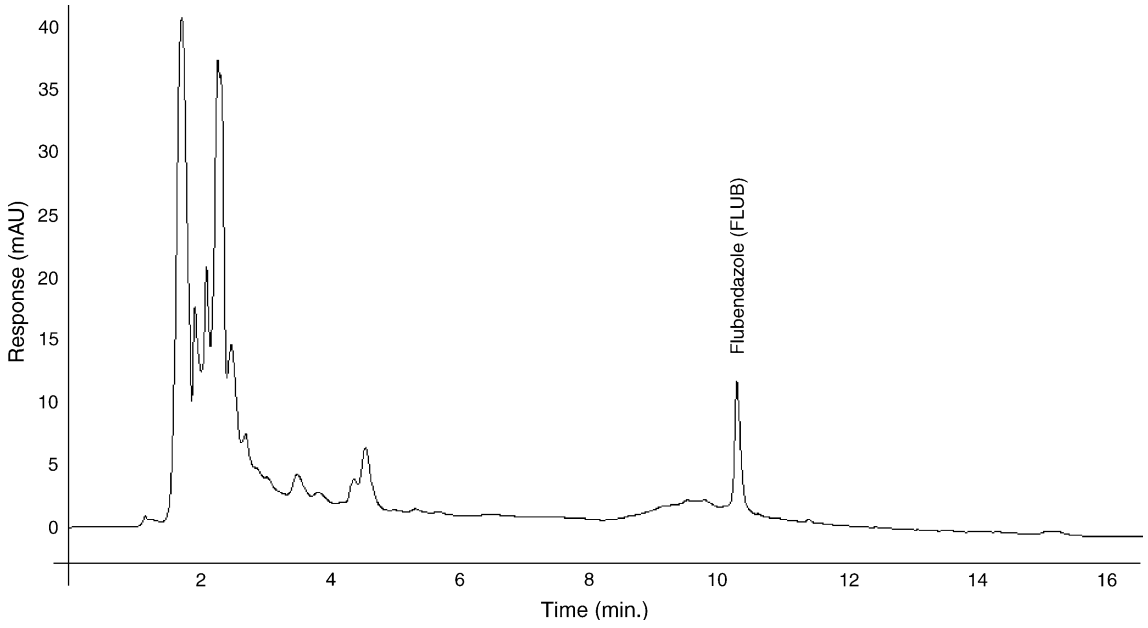

Fig. 3. HPLC chromatogram of a commercial feed sample with a declared concentration of FLUB (30 mg kg^{-1}).

Table 3. The agreement between the declared amount of FLUB and the found concentration was good and the R.S.D.s was below 3%. Fig. 3 shows the chromatogram of the commercial feed sample in which the declared concentration of FLUB was 30 mg kg^{-1}.

4. Conclusions

The data reported show that the mentioned method is suitable for the analyses of FLUB and FEBA in poultry and swine feeds with good recovery, specificity and repeatability. Furthermore the described assay offers a number of advantages in terms of simplicity, time and cost of analysis.

These factors indicate that this method is suitable for routine analysis of FLUB and FEBA both in medicated feeds and cross-contaminated ones.

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