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Optimal Condition for Determination of Zinc Bacitracin, Polymyxin B, Oxytetracycline and Sulfacetamide in Animal Feed by Micellar Electrokinetic Capillary Chromatography

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Optimal Condition for Determination of Zinc Bacitracin, Polymyxin B, Oxytetracycline and Sulfacetamide in Animal Feed by Micellar Electrokinetic Capillary Chromatography

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3 **Optimal Condition for Determination of Zinc Bacitracin,**
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6 **Polymyxin B, Oxytetracycline and Sulfacetamide in Animal Feed**
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9 **by Micellar Electrokinetic Capillary Chromatography**
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15 *Rade Injac^{1*}, Ales Mlinaric¹, Vukosava Djorjevic-Milic², Katarina Karljivic-*
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ABSTRACT

A separation of zinc bacitracin, polymyxin B, oxytetracycline and sulfacetamide in animal feedstuffs by micellar electrokinetic capillary chromatography (MEKC) was developed. The running buffer was 20 mmol L⁻¹ borate 20 mmol L⁻¹ phosphate, pH 8.4, containing 20 mmol L⁻¹ sodium dodecylsulphate and 10 % (v/v) methanol. MEKC was performed at 25°C, the applied voltage was 25 kV and running pressure of 10 mbar was applied. Simultaneous UV detection for all analytes was at 215 nm. The method was validated for specificity, accuracy, linearity, precision and robustness. It was shown to be specific, accurate (recoveries were 99.7 ± 0.3, 99.9 ± 0.9, 99.8 ± 1.0 and 99.5 ± 0.4, respectively for oxytetracycline, sulfacetamide, polymyxin B and zinc bacitracin spiked samples of feed for cow, pigs, chicken and cattle), linear over the tested range (correlation coefficients ≥ 0.9987), and precise (RSDs below 1.8 % for each analyte). The method was applied to determine zinc bacitracin, polymyxin B, oxytetracycline and sulfacetamide as additives in animal feed.

KEYWORDS: Animal feed additives, Zn bacitracin, oxytetracycline, polymyxin B, sulfacetamide, micellar electrokinetic capillary chromatography (MECK)

INTRODUCTION

Feed additives are intended to improve feed quality, nutritional aspects, animal health and animal performance. According to Regulation (EC) No. 1831/2003 (European Union, 2003) there are a wide range of substances considered as feed additives and classified as technological, organoleptic, nutritional and zootechnical (i.e. increasing animal production or performance).

Antibiotics have been used in the rearing of food-producing animals in livestock farm industries as active disease treatment agents for prophylactics and in cases of pulmonary, urinary and digestive infections as feed additives. The use of these drugs to control and treat animal disease and to promote fast, more efficient growth of livestock is a common practice. Improper use, as well as the systematic administration of such additives at sub-therapeutic doses can lead to the development of antibiotic-resistant bacteria that can be transferred to humans (Witte, 1998; Smith and Coast, 2002). Since 1999, the European Union has forbidden the use of those antibiotics as additive in animal feed (Molterer, 1998), but they are still in use in non EU countries.

Recently, Serratosa *et al.*, (Serratosa *et al.*, 2006) published an overview of the presence of residues from veterinary medicinal products, growth-promoting agents and performance enhancers in food-producing animals, as a result of legally or illegally administration of these substances, as well as the current

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3 situation in EU and practical challenges for uncovering illegal uses and
4 prevention public health risks.
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10 In order to carry out an efficient official controls of the possible illegal use of
11 these drugs in feeding stuffs, reliable methods of analysis need to be available
12 with efficient separation and high sensitivity. Oka *et al.*, (2000) reviewed the
13 literature for the assay of tetracycline antibiotics in food, among which
14 oxytetracycline, tetracycline, chlortetracycline and doxycycline are commonly
15 applied to food-producing animals. They have summarized the application of
16 thin-layer chromatography, capillary electrophoresis, and HPLC for the
17 separation and detection, as well as extraction and clean up procedures for real
18 samples.
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34 Capillary electrophoresis (CE) has become a very useful method for
35 pharmaceutical analysis and in comparison with HPLC has many advantages
36 such as: high resolution, speed, the exceptionally small sample volume required
37 and low content of organic solvents in running buffer and short run time for the
38 separation. Oka *et al.*, (2000) indicated that CE had less applications in the
39 analysis of drugs in food due to low sample injection volume, and on the other
40 hand authors pointed out that due to properties of tetracycline's these bind with
41 silanol groups in the stationary phase interfere with the establishment of a simple
42 analytical method.
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3 Recently, Kowalski *et al.*, (2003) proposed a CE method with UV detection for
4 eight of the most frequently used antibiotics (among those was doxycycline) with
5 detection limit of drug residue in tissues at level below 20 $\mu\text{g}/\text{kg}$. One of the
6 commonly used CE modes is micellar electrokinetic capillary chromatography
7 (MEKC), which is efficient for the separation of both ionic and neutral analytes.
8 Success of the separation is based mainly on appropriate selection of the
9 surfactant. The results of MECK application for Zn Bacitracin and nystatin
10 separation and assay in animal feed, recently developed in our laboratories (Injac
11 *et al.*, 2006) demonstrated that this technique could be the alternative to HPLC
12 method, but also the simple extraction procedure was proposed. For zinc
13 bacitracin in animal feed Capitán-Vallvey *et al.* (2001 and 2002) proposed HPLC
14 methods with UV and fluorescence detection, but with difficult extraction
15 procedures using LLE as well as SPE techniques.
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36 In the continuation of our research studies we selected, beside Zn Bacitracin (Zn
37 BAC), oxytetracycline (OXY), polymyxin B (POL B) and sulfacetamide (SUL) to
38 investigate the separation applying MECK with the similar extraction procedure
39 described in previously published paper (Injac *et al.*, 2006). OXY, SUL, Zn BAC
40 and POL B have been determined in pharmaceutical formulations by CZE and
41 MEKC (Gallego and Arroyo, 2002; Gallego and Arroyo, 2003a; Gallego and
42 Arroyo, 2003b; Gallego and Arroyo, 2003c; Gallego and Arroyo, 2003d; Mamani
43 *et al.*, 2006; Nozal *et al.*, 2004). Kang *et al.* (Kang *et al.*, 2001) have used MEKC
44 to separate bacitracin components, with Brij 35 and PAPS as surfactants.
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3 Counter-current chromatography and high-performance liquid chromatography
4 were used for the separation of Zn-bacitracin (Oka *et al.*, 1998; Capitan-Vallvey
5 *et al.*, 2002; Sin *et al.*, 2005). Tetracyclines and sulfonamides were analysed by
6 liquid chromatography-electrospray ionization tandem mass spectrometry, as
7 well as with HPLC-UV, LC/MS/MS in wastewaters, pharmaceuticals and food
8 (Yang *et al.*, 2005; Zhao *et al.*, 2004; Mandens *et al.*, 2004; Rao *et al.*, 1999).
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20 Zn BAC interferes with bacterial cell wall synthesis by blocking the function of the
21 lipid carrier molecule that transfers cell wall subunits across the cell membrane. It
22 is active against many Gram-positive bacteria. Acquired bacterial resistance to
23 bacitracin rarely occurs (Martindale, 2005). Oxytetracycline is bacteriostatic
24 antibiotic with a wide spectrum of activity and has been used in the treatment of a
25 large number of infections caused by susceptible organisms. Polymyxin B and
26 the other polymyxin antibacterials act primarily by binding to membrane
27 phospholipids and disrupting the bacterial cytoplasmic membrane. The use of
28 sulfacetamide and other sulfonamides has been limited by the increasing
29 incidence of resistant organisms (Wang *et al.*, 2006). Their main use has been in
30 the treatment of acute, uncomplicated urinary-tract infections, particularly those
31 caused by *Escherichia coli* (Martindale, 2005).
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50 Selected analytes, OXY, SUL, Zn-BAC and POL used as additives in animal feed
51 at levels 100-200 mg kg⁻¹, 100 mg kg⁻¹, 50-250 mg kg⁻¹ and 50-150 mg kg⁻¹,
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3 respectively (Swine antibiotics and feed additives, 2006; Frost & Sullivan, 2005;
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6 National Department for Veterinary Science, 2006).
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10 The aim of this paper was to establish the optimal conditions for MECK
11 separation and assay using the model mixture of OXY, Zn BAC and POL B in
12 spiked feedstuff samples, antibiotics belonging to different cases of antibiotics, in
13 combination with SUL as these can be found as feed additives. The model
14 mixture is also of interest since, polymyxins have been reported to demonstrate
15 antimicrobial synergy with a variety of other drugs, including chloramphenicol,
16 tetracycline, and the sulfonamides and trimethoprim (Martindale, 2005), and as
17 such the prophylactics in food could be covered with these representative drugs.
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32 **MATERIALS AND METHODS**

33 **Apparatus**

34 The HP^{3D} Capillary Electrophoresis system (Hewlett Packard, Waldbronn,
35 Germany) with a diode-array detector, controlled by HP ChemStation software,
36 was used to perform MEKC. Compounds were separated on a 48 cm (40 cm to
37 the detector) x 50 μm i.d. fused silica capillary (with bubble cell, 150 μm) (Agilent,
38 Waldbronn, Germany).
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48 A Crison MicropH 2002 pH meter (Barcelona, Spain) was used for pH
49 measurement.
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55 **Reagents and solutions**

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3 All solvents and reagents were of analytical grade unless indicated otherwise.
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5 Samples solutions were prepared with deionized water (Milli-Q-quality).
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7 Sulfacetamide sodium was obtained from Vetprom (Belgrade, Serbia) and Zn-
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9 bacitracin from Sigma (Deisenhofen, Germany). The quality of the both complied
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11 with BP requirements. Oxytetracycline hydrochloride and polymyxin B sulphate
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13 were obtained from Sigma (Deisenhofen, Germany) and Fluka (Buchs,
14
15 Switzerland), respectively (USP quality).
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22 Animal diet and feedstuff mixtures for cow K-19 (protein min 19 %, crude fat max
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24 15 %, ash max 15 %, crude fiber max 10 %, calcium 0.8 %, phosphorus 0.45 %,
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26 sodium 0.25 %, moisture max 13.5 %, vitamins A, D₃, E, zinc, manganese,
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28 selenium, cobalt, copper, iron and iodine), young cattle TL-TIP (protein min 14 %,
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30 ash max 10 %, crude fiber max 12 %, moisture max 13.5 %, vitamins A, D₃, E,
31
32 zinc, manganese, selenium, cobalt, copper and magnesium), pigs from 25 to 60
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34 kg BEK-1 (protein 17 %, ash max 8 %, crude fiber max 7 %, moisture max 13.5
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36 %, vitamins A, D₃, E, B-complex, zinc, selenium, manganese, iodine, cobalt,
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38 copper, iron and antioxidants Sanox), chicken feed NSK-1 (protein min 15 %,
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40 moisture max 13.5 %, crude fiber max 8 %, ash max 12 %, vitamins A, D₃, E, K₃,
41
42 B-complex, zinc, selenium, manganese, iodine, cobalt, copper, iron, carophyll red
43
44 and antioxidants Sanox), and minerals and vitamins for cattle BOVISAL (calcium
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46 16 %, phosphorus 11 %, sodium 10 %, magnesium 0.6 %, moisture max 7 %,
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48 vitamins A, D₃, E, zinc, manganese, selenium, iodine, cobalt and copper), were
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50 manufactured by JATA EMONA (Ljubljana, Slovenia).
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6 Buffer solutions were prepared by dissolving the appropriate amount of NaH_2PO_4
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8 (20 mmol L^{-1}) and $\text{Na}_2\text{B}_4\text{O}_7$ (20 mmol L^{-1}) in deionized water and the pH was
9
10 adjusted to 8.4 with HCl. $\text{Na}_2\text{B}_4\text{O}_7 \times 10\text{H}_2\text{O}$ was p.a. from Kemika (Zagreb,
11
12 Croatia) and $\text{NaH}_2\text{PO}_4 \times \text{H}_2\text{O}$ was p.a. from J.T. Baker Inc. (Phillipsburg, USA).
13
14 Sodium dodecylsulphate (SDS) was from Riedel Line AG (Seelze, Germany).
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16 The background electrolyte (BGE) was 20 mmol L^{-1} borate + 20 mmol L^{-1}
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18 phosphate buffer, pH 8.4, containing 20 mmol L^{-1} SDS and 10 % (v/v) methanol.
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24 **Preparation of standard stock solutions**

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27 Stock solutions of antibiotics were prepared by weighing 50 mg of the drugs and
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29 dissolving in 50 mL methanol - deionized water (1:1; v/v). The stock solutions
30
31 were diluted with running buffer to obtain the concentration ranges required
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33 (linearity was check in range of $0.1 - 100 \text{ mg kg}^{-1}$ for each drug).
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39 **Sample preparation and extraction**

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41 Spiked animal feed were prepared by grinding 500 g of feedstuff and adding 50
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43 mg of each of the antibacterial. Blank feed or spiked feed with added antibiotics,
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45 were weighed (100 g) and extracted with methanol (5 x 20 mL), first by shaking,
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47 then in an ultrasonic bath for 15 min. The extracts were combined, filtered (0.22
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49 μm), transferred to 100 mL volumetric flask, and made up with methanol.
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52 Different known volumes were placed in 1.5 mL calibrated vials and diluted to
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54 volume with running buffer.
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Operating conditions

The capillary was conditioned prior to its first use by flushing with 0.1 mol L⁻¹ NaOH for 20 min and then with water for 10 min. The capillary was conditioned, in the optimized method, at the beginning of each day with methanol under high pressure for 3 min, than rinsed for 2 min with 0.1 mol L⁻¹ NaOH and 3 min with background electrolyte. This was followed by hydrodynamic sample injection at 600 mbars. Separations were performed at 25 kV and 25°C (under applied pressure of 10 mbar) in 12 min; under these conditions the current was 97-98 µA. UV detection was at 215 nm.

RESULTS AND DISCUSSION

Preliminary studies

Preliminary investigations of Zn-BAC, SUL, OXY and POL B were carried out, to optimize the separation. MEKC methods were published for the determination of hydrocortisone and its most important associated compounds (including Zn-BAC, POL B and OXY) in topical pharmaceutical preparations (Gallego and Arroyo, 2002; Gallego and Arroyo, 2003c) SUL were determined in pharmaceuticals by MEKC with others associated compounds (Gallego and Arroyo, 2003a). Some modifications of the SDS concentration, organic modifier and applied pressure were assessed to obtain a shorter run time and better resolutions in separation of all analytes from animal feed.

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3 The published literature (Gallego and Arroyo, 2002; Gallego and Arroyo, 2003a)
4 pointed out the effect of pH values (6 - 11) of phosphate-borate (1:1) buffer,
5 buffer component concentrations (10 to 60 mmol L⁻¹) and effect of organic
6 modifier (methanol, acetonitrile; 3 – 12 %). In our research the presence of 10 %
7 methanol in the electrolyte resulted, in well resolved peaks and the shoulders
8 disappeared. The results demonstrated the separation is better when the pH is
9 between 8 and 9. A 40 mmol L⁻¹ (20 mmol L⁻¹ borate/20 mmol L⁻¹ phosphate
10 buffer) concentration was considered suitable for its good resolution and peak
11 shape. Higher concentration of buffer resulted in peak broadening.
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27 Running voltages effects in the range 5-30 kV were tested using a background
28 electrolyte of 20 mmol L⁻¹ borate/20 mmol L⁻¹ phosphate buffer, pH 8.4,
29 containing 10-80 mmol L⁻¹ sodium dodecylsulphate (SDS) and 10% methanol,
30 without running pressure, at 25°C. The best results were at 25 kV, and an
31 acceptable level of baseline noise was achieved by performing experiments at
32 25°C.
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44 The influence of SDS in the electrolyte on the migration time (without running
45 pressure) is very significant. The results show that the SDS concentration
46 dramatically affects the migration time of the Zn-BAC and POL B, but not that of
47 SUL and OXY. A concentration of 70 mmol L⁻¹ was selected for the experiment
48 as to give the best resolution, but without a short analysis time (20 min) and
49 without good symmetry and widths of Zn-BAC and POL B peaks.
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3 Running pressure was tested in the range 5-30 mbar, using the above
4 experimental conditions. Migration times were decreased with increasing running
5 pressure, and the value of 10 mbar was selected as optimum. It gives the best
6 resolution and symmetric peaks in all cases, and also the analysis time is
7 shorter. Under such experimental conditions the concentration of 20 mmol L⁻¹
8 SDS was selected as the most advantageous. It gave the best separation without
9 broadened and deformed peaks, and acceptable resolution for Zn-BAC and POL
10 B (Figure 1).
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24 The best results were with applying pressure of 10 mbar at 25 kV. UV detection
25 was at 215 and 254 nm for Zn-BAC, SUL and OXY, 205 and at 215 nm for POL
26 B, for simultaneous detection 215 nm was selected. The electropherograms
27 obtained in the separation under selected conditions are presented in Figure 2. It
28 is remarkable that all peaks have good resolutions in a run time of 12 min (OXY
29 3.1; SUL 3.7; POL B 9.3; Zn-BAC 10.6). The conditions of MECK with SDS was
30 exceptionally suitable for OXY, since it is eluted as the first peak without any
31 interferences, confirming that this technique could be more efficient in
32 comparison to HPLC, since Oka et al. (2000) pointed out that EDTA and oxalic
33 acid should be used during each analytical step (extraction, clean up and HPLC
34 separation). Our recently published paper (Injac *et al.* 2007) denoted sufficient
35 difference in migration time for two tetracycline antibiotic OXY and doxycycline.
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55 **Validation of the method**

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3 The characteristics and the procedures used for validation were those described
4 in USP 24 (USP 24, 2000), the International Conference of Harmonization (ICH)
5 Guidelines (ICH Q2A, 1995; ICH Q2B, 1997) and other literature (Heyden et al.,
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The characteristics and the procedures used for validation were those described in USP 24 (USP 24, 2000), the International Conference of Harmonization (ICH) Guidelines (ICH Q2A, 1995; ICH Q2B, 1997) and other literature (Heyden et al., 1999; Shabin, 2003; Brown et al., 2001; Toro et al., 2004; Altria and Chanter, 1993; Injac et al., 2007).

Selectivity

The selectivity of the method was investigated by observing interfering peaks from matrix present in the feedstuffs. Five different feedstuff mixtures were tested for the interferences. There was no interference in MEKC results by the feed's ingredients in any of the tested mixtures, which indicates that the method is selective.

Linearity

The linearity of the assay was determined by analysis of a series of standards at five different concentrations in range of 0.1 to 100 mg kg⁻¹ for each compound.

The linearity of calibration curves (peak area vs. concentration) for OXY, SUL, POL B and Zn-BAC over the concentration ranges of 6.5 – 77.2 mg kg⁻¹ for OXY, 0.3 – 84.5 mg kg⁻¹ for SUL, 18.5 – 85.0 mg kg⁻¹ for POL B and 12.1 – 62.4 mg kg⁻¹ for Zn-BAC gave correlation coefficients of 0.9994, 0.9997, 0.9987 and 0.9991, respectively.

Limit of detection (LOD) and limit of quantification (LOQ)

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3 LODs and LOQs were estimated by the baseline noise method. Baseline noise
4 was evaluated by recording the detector response over a period of ten times the
5 peak width. LOD and LOQ, respectively, were defined as the analyte
6 concentrations resulting in peaks of height three and ten times the baseline noise
7 level ((ICH Q2A, 1995; ICH Q2B, 1997; Heyden et al., 2001). Thus, LODs/LOQs
8 are 1.6/6.2, 0.1/0.3, 6.0/17.5 and 3.7/12.3 mg kg⁻¹ for OXY, SUL, POL B and Zn-
9 BAC, respectively.
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20 21 22 **Accuracy**

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24 The accuracy of the method was determined by analyzing a solution of known
25 concentration (working standard solution – spiked feed samples with standards)
26 and comparing the measured and known values. Fifteen determinations were
27 carried out for five concentration levels. The mean recovery was 99.7 ± 0.3, 99.9
28 ± 0.9, 99.81 ± 1.0 and 99.5 ± 0.4 for OXY, SUL, POL B and Zn-BAC,
29 respectively, proving a good accuracy of the method.
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40 41 **Precision**

42 Precision can be measured as repeatability, reproducibility, and intermediate
43 precision. In this work only repeatability and intermediate precision were studied.
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48 **Repeatability**

49 A repeatability test was performed to determine intra-day variation in corrected
50 areas and migration times. Working solutions of concentrations 20, 40 and 60 mg
51 L⁻¹ (relative to 20, 40 and 60 mg kg⁻¹) were analyzed ($n = 6$). The RSD values for
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3 migration times (0.29 % for OXY, 0.13 % for SUL, 0.94 % for POL B and 0.82 %
4 for Zn-BAC) and for peak areas (0.92-1.76 % OXY, 0.57-0.77 % SUL, 1.12-1.53
5 % POL B and 0.85-1.72 % Zn-BAC) indicate that the repeatability of the method
6 is acceptable.
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11 ***Intermediate Precision***

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13 Intermediate precision was evaluated over three days by performing working
14 solutions of concentrations 20-50 mg L⁻¹ (relative to 20-50 mg kg⁻¹). These were
15 injected on each of three days under the same conditions and the results were
16 used for the repeatability study. When stored in the dark refrigerated, the
17 recovery ranged from 100.2 to 99.1 % over three days. The RSD values (0.12-
18 0.34 % for OXY, 0.09-0.25 % for SUL, 0.39-0.83 for POL B and 0.42-0.81 % for
19 Zn-BC) indicate that the intermediate precision is acceptable. When they were
20 stored at room temperature in sunlight, decreasing recovery values from 100.15
21 to 94.81 % for Zn-BAC, from 100.40 to 95.03 % for POL B and from 100.01 to
22 92.98 % for OXY were observed for the standards in BGE.
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41 **Robustness**

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43 The optimum MEKC conditions set for this method have been slightly modified in
44 order to evaluate the robustness. The effects of different concentrations of SDS
45 (20 ± 1 mmol L⁻¹) and organic modifier (10 ± 0.5 % methanol) in the mobile
46 phase, as well as the effects of buffer pH (8.4 ± 0.06), capillary temperature (25 ±
47 5°C), applied pressure (10 ± 1 mbar), running voltage (25 ± 1 kV) and detection
48 wavelength (± 3 nm), and were determined. The design applied was the
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fractional factorial design (Heyden et al., 2001). No significant variations in accuracy, specificity and precision were found over the tested ranges, which indicated that the method conditions are robust.

Stability of antibiotics

The stability of OXY, SUL, POL B and Zn-BAC in acidic (pH < 5) and basic (pH > 10) solutions was checked in test samples at room temperature for 24 and 48 h and the recoveries were 98.2 ± 2.3 % at 24 h and 95.8 ± 3.8 % at 48 h. The stability in the BGE was also checked at 24 h. Recoveries of each compounds were ≥ 99.3 %. It was indicating good stability.

Application

The present method was tested by determining OXY, SUL, POL B and Zn-BAC in animal feedstuffs. When analyzing spiked commercial products, the amounts and recoveries obtained were determined by comparing the results with a standard solution containing the same concentration as expected in the spiked commercial products. The results presented in Table I show good agreement between the claimed and found values. Recovery values also confirmed that, due to the sufficient solubility of investigated compounds, extraction procedure was efficient.

According to LOQ values for each analyte, the minimum quantifiable amounts with the proposed method are 6.2, 0.3, 17.5 and 12.3 mg kg⁻¹ (ppm) of OXY,

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3 SUL, POL B and Zn-BAC, respectively in feed. The sensitivity of the proposed
4 method is acceptable in relation to the efficiency levels of OXY, SUL, POL B and
5 Zn-BAC as additives and can be utilized for routine control of animal feedstuffs.
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8 The obtained results are in correlation that CE is already an official method in
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10 USP will also be valuable method as the alternative to HPLC (S. Görög, 2007)
11 and the precision of MECK (Injac *et al.*, 2007) confirmed the applicability in
12 routine quality control.
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22 The advantage of proposed method over the HPLC and TLC methods described
23 in literature for analysis OXY, SUL, POL B and Zn-BAC in different samples is its
24 lower running costs and higher environmental friendliness. In the proposed
25 method, 20-30 analyses with MEKC require 2 mL of phosphate/borate buffer
26 containing SDS and 10 % (v/v) methanol, while 20 analyses by HPLC require
27 300 to 1000 mL of mobile phase with different proportion of most commonly used
28 organic solvent (methanol, acetonitrile, acetone, THF). The advantage of LC
29 (MS; MS/MS) methods over MECK is evident for the analysis of residues in
30 samples of different tissues (Bogialli *et al.*, 2003).
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46 CONCLUSION

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48 The MEKC method presented here is a useful technique for rapid separation
49 (within 12 min) of zinc bacitracin, polymixin B, oxytetracycline and sulfacetamide
50 at low concentration of surfactant (SDS 20 mmol L⁻¹) and pH 8.4 (phosphate-
51 borate buffer). A pressure of 10 mbar was applied and it gives the best resolution
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3 and symmetric peaks in all cases, special for POL B and Zn-BAC. This system
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5 was also applied successfully to their identification and assay in animal feedstuff,
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7 with different matrixes, spiked with zinc bacitracin, polymixin B, oxytetracycline
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9 and sulfacetamide.
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12 13 14 15 **Acknowledgment**

16
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18
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50 **Figure captions (R. Injac et al.)**

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52 **Figure 1.**
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3 Effect of SDS concentration on resolution with a applied pressure of 10 mbar.
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5 The electrolyte solution was 20 mmol L⁻¹ phosphate + 20 mmol L⁻¹ borate buffer,
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8 pH 8.4, containing 10% methanol, and the temperature and voltage were 25°C
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10 and 25 kV, respectively.
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12 **Figure 2.**

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15 Electropherograms obtained **A)** 100 mg kg⁻¹ of Zn-BAC, POL B, SUL and OXY
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17 standards, and **B)** 50 mg kg⁻¹ of Zn-BAC, POL B, SUL and OXY from spiked
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19 animal feedstuff; under the optimized conditions, at 215 nm. The BGE was 20
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21 mmol L⁻¹ phosphate + 20 mmol L⁻¹ borate buffer, pH 8.4, containing 20 mmol L⁻¹
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23 SDS and 10% methanol, the temperature and voltage were 25°C and 25 kV,
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25 respectively with applied pressure of 10 mbar.
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29 **Table I.**

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31 Application results for spiked feedstuff samples.
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Table I R. Injac et al.

Tested Sample (<i>n</i> = 10)	Amount expected (mg)	OXY Amount found (mg)	OXY Recovery (%)	SUL Amount found (mg)	SUL Recovery (%)	POL B Amount found (mg)	POL B Recovery (%)	Zn-BAC Amount found (mg)	Zn-BAC Recovery (%)
K-19	50	49.9±0.1	99.8	48.9±0.5	97.8	48.9±0.4	97.8	49.3±0.3	98.6
TL-TIP	50	49.9±0.2	99.8	49.8±0.3	99.6	48.8±0.3	97.6	49.2±0.4	98.4
BEK-1	50	50.0±0.1	100.0	49.9±0.1	99.8	49.3±0.8	98.6	50.2±0.3	100.4
NSK-1	50	49.8±0.1	99.6	49.7±0.4	99.4	49.5±0.5	99.0	50.3±0.1	100.6
BOVISAL	50	50.1±0.3	100.2	49.8±0.2	99.6	49.1±0.6	98.2	49.7±0.7	99.4

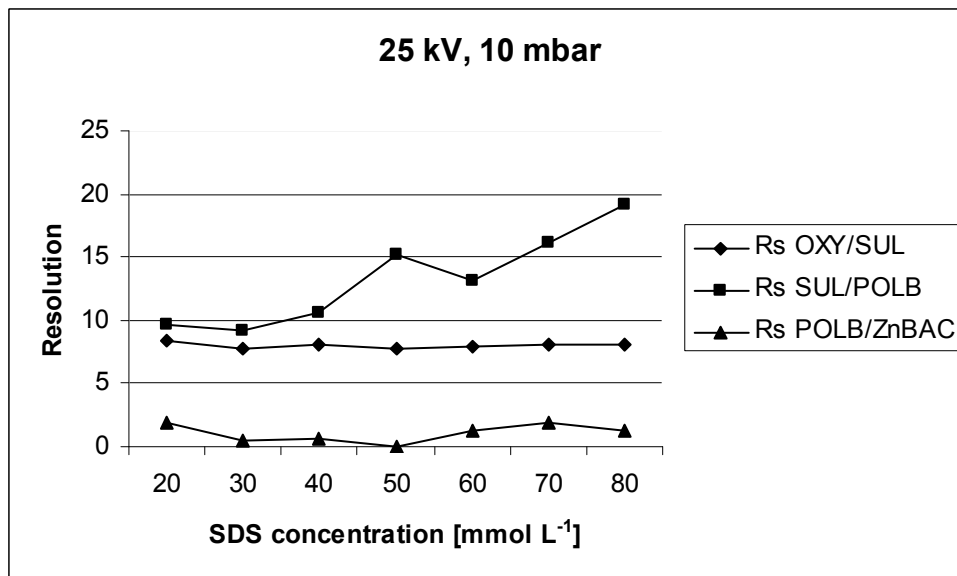
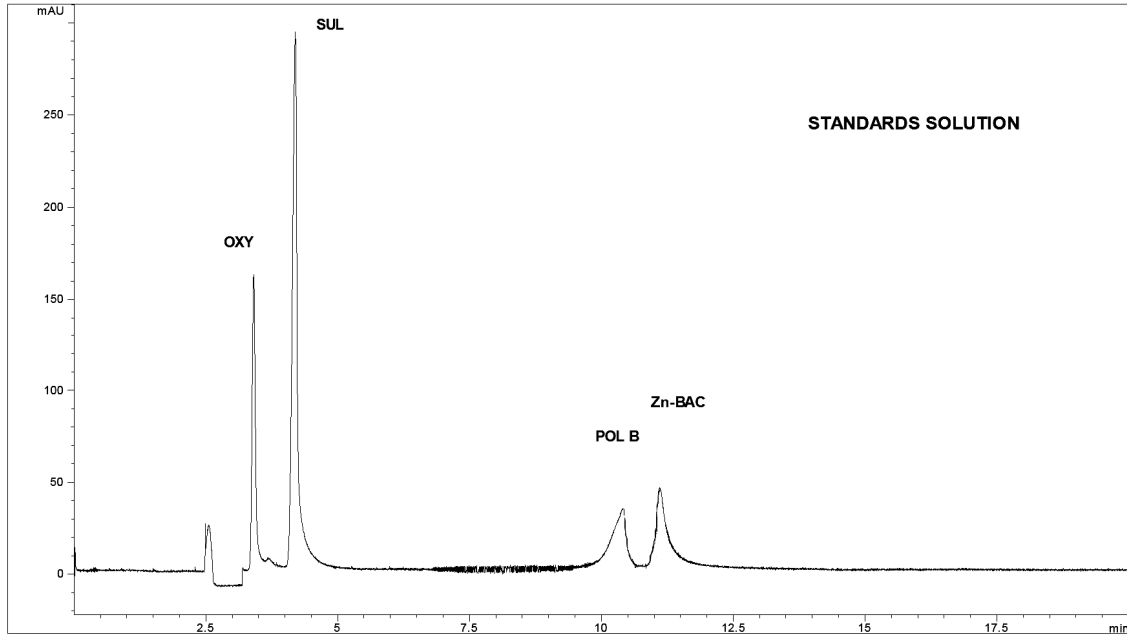
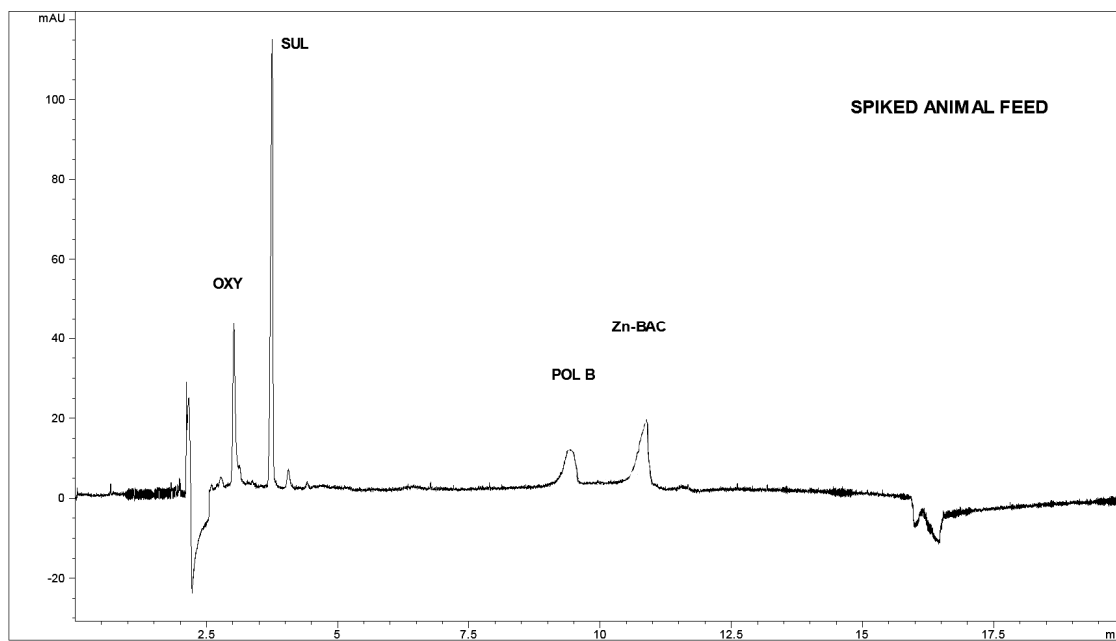
Figure 1 R. Injac et al.

Figure 2A R. Injac et al.



Review Only

Figure 2B R. Injac et al.



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