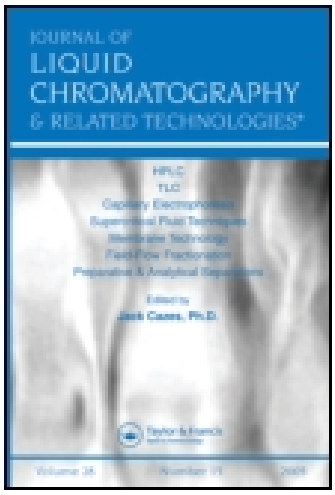

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Development and Validation of a CD-MEKC System for the Simultaneous Determination of Dihydrostreptomycin Sulfate and Two Benzylpenicillin Salts

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Development and Validation of a CD-MEKC System for the Simultaneous Determination of Dihydrostreptomycin Sulfate and Two Benzylpenicillin Salts

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A CD-MEKC system using sodium dodecyl sulfate and sulfated-β-cyclodextrin to achieve the simultaneous separation of Penicillin G procaine, Penicillin G benzatine and dihydrostreptomycin sulfate in pharmaceutical and veterinary dosage forms was developed. Several parameters were evaluated, including buffer type and concentration, cyclodextrin type and concentration, and organic solvent and instrumental parameters. The optimized system consisted of 50 mM SDS, 1% sulfated-ß-cyclodextrin, and 10 mM pH 9.0 borate buffer. Capillary temperature was kept at 35°C, and an electrophoretic system was operated under positive polarity at a constant voltage of 18 kV. Validation parameters such as specificity, linearity, LOD, and LOQ, precision, accuracy, and robustness were assayed. Therefore, the developed method was found to be appropriate for quality control of Penicillin G procaine, Penicillin G benzatine, and dihydrostreptomycin sulfate.

Keywords: chiral micellar capillary electrophoresis, dihydrostreptomycin sulfate, Penicillin G benzatine, Penicillin G procaine, quality control

Introduction

Nowadays, multiantibiotic therapy is one of the most common practices in the treatment of many infections, due to the advent of new antimicrobial agents added to the development of microbial resistance. Multiantibiotic therapy could have different objectives such as broadening the spectrum to guarantee efficacy and multimicrobial infections to prevent the development of resistance and to exhibit a synergic effect.[1,2] One of the most used combinations is beta-lactams and aminoglycosides such as penicillin G salts and dihydrostreptomycin sulfate (DHS), respectively.

Antimicrobial agents have revolutionized human and also veterinary medicine through the provision of an effective and affordable tool for the treatment and prophylaxis of bacterial infectious diseases. In addition, antimicrobial drugs have been used for more than fifty years for their growth stimulatory effects, when they are administered to healthy animals at dosage rates lower than those that are considered effective for the treatment of animals with clinical disease.[3]

Penicillin has been widely used since it was discovered and still today is the most important group of antibiotics. Several types of penicillin have different antimicrobial spectrum or activity, inhibiting the last step on the synthesis of peptidoglycan of the bacterial wall.[4]

Penicillin G (PG) (Figure 1a) is a narrow spectrum penicillin commonly used in the treatment of many diseases. In pharmaceutical preparation, it is available in the form of potassium or sodium (water soluble) salts; also, it is supplied as procaine (Pr) (Figure 1b) or benzathine (Bz) (Figure 1c) (insoluble in water) salts.[5] Although sodium salt of PG reaches a maximum concentration after 15 or 30 min of administration in plasma, penicillin G benzathine (PGBz) and penicillin G procaine (PGPr) are long-lasting penicillins. In order to achieve the plasma concentration profile optimal for efficacy, several strategies may be adopted; one of these is the use of formulations combining PGPr and PGBz, where the associated release profile gives an appropriate plasma concentration-time profile for target pathogens.

DHS belongs to the aminoglycosides group of ATB, which are useful therapeutic agents due to their high efficacy against Gram negative organisms. Chemically, they are polycationic amino sugars produced by *Streptomyces sp, Micromonospora sp and Bacillus sp* (Figure 1d).

Current testing methods for the determination of PG (and its salts) include iodometric and potentiometric titrations as well as liquid chromatographic and microbiological assays.[6–8] Given the lack of strong UV chromophores or fluorophores in DHS,

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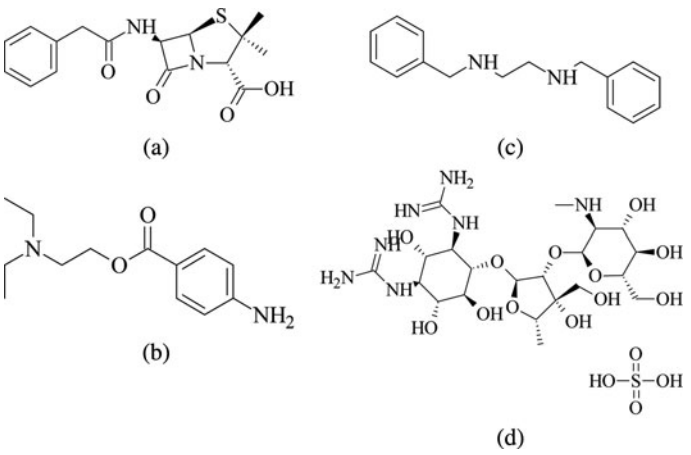

Fig. 1. (a) Chemical structure of penicillin G, (b) dihydrostreptomycin sulfate, (c) benzathine, and (d) procaine.

the assay is a critical point for its determination. Analytical methods applied to the determination of DHS include separation techniques such as HPLC or capillary zone electrophoresis (CZE), although most pharmacopoeias recommend the microbiological determination of DHS.[9] In the HPLC method described by Adams et al.,[10] an ion-pair reversed phase with UV detection at 195 or 205 nm can be applied for the analysis of DHS. DHS and related substances can also be analyzed by CZE with direct UV detection at 205 nm, using borate complexation at pH 10.25[11,12] or by indirect UV detection using the anionic mode and a reversed electroosmotic flow (EOF) by addition of fluorochemical surfactant FC 135 to the background electrolyte (BGE).[13]

Capillary electrophoresis (CE) is actually a wide group of electroseparation techniques based on different separation modes its applications depend on the complexity of the sample, the nature of its components, or the intended application and the nature of the analytes, and each CE mode will provide various advantages for the separation and detection of different substances. Micellar electrokinetic chromatography (MEKC) is a mode of CE presented for the first time by Terabe et al.,[14] which extends the applicability of CZE to neutral analytes and improves separation between analytes with quite similar electrophoretic mobilities, which cannot be separated using simple free solution CE. The separation principle of MEKC is based on the differential partition of the analytes between micelles and water together with their electrophoretic mobilities, whereas CZE is based solely on the differences between the electrophoretic mobility of each analyte. Thereby, the greatest advantage of MEKC over CZE is the application in the separation of both ionic and neutral analytes. As MECK is often applied to the separation of chemically related compounds, generally showing similar physicochemical properties, it is a common practice to improve the selectivity of a MECK system with the use of buffer additives such as organic modifiers and cyclodextrins.[15]

Up to now, CE methods in two of its modes, CZE and MEKC with cyclodextrins (CD-MEKC), for the analysis of PG, Pr, and DHS and the analysis of benzylpenicillin salts, respectively, have been described in literature.[5,9] Nevertheless, no method for the simultaneous determination of Pen G, procaine, benzatine, and DHS has been previously described by any methodology.

The aim of this work is to develop and validate a CD-MEKC method for the simultaneous analysis of PGPr, PGBz, and DHS and demonstrate its suitability to the quality control routine analysis. As an example, the method was applied to a suspension for veterinary use with contents of the three ATB. The content of PGPr and PGBz were indirectly calculated through the quantification of Pr and Bz in the formulation.

Experimental

Chemicals and Reagents

Penicilin G procaine (PGPr) (2-(diethylamino)ethyl 4-aminobenzoate;(2S,5 R,6 R)-3,3-dimethyl-7-oxo-6-[(2-phenylacetyl) amino]-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid; hydrate), Penicilin G benzathine (PGBz) (N,N′-dibenzylethane-1,2-diamine;3,3-dimethyl-7-oxo-6-[(2-phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid), dihydrostreptomycin (DHS)(2-[(1 R,2 R,3S,4 R,5 R,6S)-3-(diaminomethylideneamino)-4-[(2 R,3 R,4 R,5S)-3-[(2S,3S,4S,5 R,6S)-4,5-dihydroxy-6-(hydroxymethyl)-3-(methylamino)oxan-2-yl]oxy-4-hydroxy-4-(hydroxymethyl)-5-methyloxolan-2-yl]oxy-2,5,6-trihydroxycyclohexyl] guanidine) were supplied by CSPC Zhongnuo Pharmaceutical (Shijiazhuang, CO, LTD., China). Sodium dodecylsulfate (SDS), ß-cyclodextrin sulfated sodium salt, ß-cyclodextrin, 2-hydroxypropyl-ß-cyclodextrin, and tetraborate sodium salt were purchased from sigma (St. Louis, MO, USA). Methanol (HPLC grade) was purchased from Merck (Darmstadt, Germany). Water was purified in a EASY Pure RF equipment (Barnstead, Dubuque, IA, USA).

Instruments

All CE separations were performed using a P/ACE MDQ CE system (Beckman, Fullerton, CA, USA). Uncoated fused silica capillaries (Microsolv Technology, Eatontown, NJ, USA) of 50 cm (40 cm length to detector) × 75 µm id., were used. The separation was performed by a CD-MEKC system consisting of 50 mM SDS, 1% sulfated-ß-cyclodextrin, and 10 mM borate buffer at pH 9.0, which constituted the BGE. Capillary temperature was kept at 35°C, and UV detection was set at 200 nm. Samples were injected under 0.3 psi pressure for 3 seconds and electrophoretic system was operated under positive polarity at a constant voltage of 18 kV. A new capillary was conditioned by rinsing with 0.5 M potassium hydroxide for 3 min, 0.1 M potassium hydroxide for 2 min, and water during 2 min. Prior to each analysis, the capillary was washed with 0.1 M potassium hydroxide, water, and the BGE for 2 min for each one.

Standard and Sample Preparation

Stock and Standard Solution

Stock solutions of PGPr and PGBz at 1 mg/mL in methanol and DHS at 2 mg/mL in water were prepared.

Standard solutions of PGPr, PGBz at 100 µg/mL, and DHS at 200 µg/mL, respectively, were prepared by appropriate dilution

of stock solutions in dilution solvent (1 mM borate buffer with a final methanol proportion of 20%).

Sample Preparation

Due to the great difference in solubility of PGPr, PGBz, and DHS, two methods for the sample preparation were required to achieve a complete recovery. The sample contains 10.000.000 UI of PGPr (1009 UI/mg) and PGBz (1211 UI/mg), and 20% w/v of DHS according to the labeled claim. The quantification of PGPr and PGBz is through the quantification of the peaks that correspond to Pr and Bz, respectively.

Determination of PGPr and PGBz. Around 500 mg of ATB suspension were accurately weighed in a 50.0-mL volumetric flask. Approximately 40 mL of methanol were added and the solution was sonicated during 30 min and made up to volume. The 5.00 mL of the previously prepared solution was diluted with the dilution solvent in a 50.0-mL volumetric flask.

Determination of DHS. Determination of DHS proceeded as described in the aforementioned section (*determination of PGPr and PGBz*), but the first dilution was placed in water instead of methanol.

Results and Discussion

Optimization of the CE System

Electrophoretic conditions were optimized in order to obtain the best resolution with an optimal run time. Parameters such as buffer pH, type, and concentration of cylodextrin and organic solvent effect were investigated.

MEKC System

Although Pr, Bz, and DHS are positively charged at pH over 9, they cannot be separated with conventional CZE, due to the fact that in the pH range 6.0–9.2 the electrophoretic mobilities were similar to the EOF mobility, and Pr and Bz migrates together, as has been demonstrated in a further study by Pajchel et al.[5] Therefore, SDS at pH 9.0 as pseudostationary phase was tested for their separation. The resolution between the three analytes was not completely achieved; even though Pr and Bz could be resolved, DHS and procaine migrate with the same velocity. These results lead to the necessity of another BGE additive to accomplish the complete separation among all the analytes.

Table 1. Comparison of resolution values with different cyclodextrins as BGE additive

	β-cyclodextrin	Hydroxypropyl-β-cyclodextrin	Sulfated-β-cyclodextrin
DHS			
Pr	1.40	1.34	7.2
Bz	1.46	1.90	11.4

Effect of Cyclodextrin Type and Concentration

A previous report described the formation of an inclusion complex between Pr and β-cyclodextrin, including the determination of the binding constant by CE.[16] Based on this evidence, in order to obtain the best resolution among the three analytes, three different cyclodextrins were tested as BGE additives. The β-cyclodextrin and hydroxypropyl-β-cyclodextrin both at concentration of 10 mM, and sulfated-β-cyclodextrin at 1% w/v were tested. Using β-cyclodextrin or hydroxypropyl-β-cyclodextrin, the resolution values between Pr and Bz were below 2. Alternatively, with the use of sulfated-β-cyclodextrin, the resolution obtained was even four times higher (Table 1) with no significant increase in the migration times (Figure 2). Moreover, we evaluate the formation of an inclusion complex between Pr and DHS with sulfated-β-cyclodextrin based on

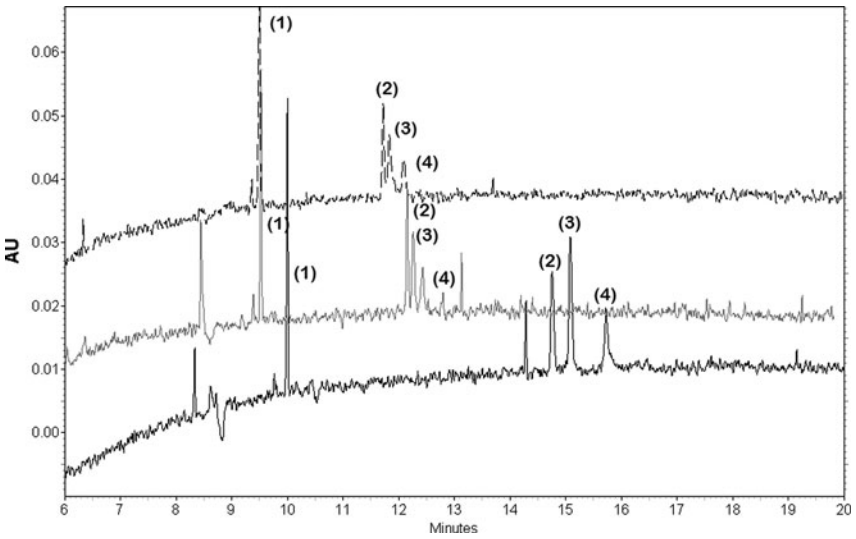

Fig. 2. Electropherogram of a standard solution of DHS, PGPr, and PGBz in three different BGE systems consisting of 10 mM pH 9.0 borate buffer with (a) 10 mM ß-cyclodextrin, (b) 10 mM ß-hydroxypropil-ß-cyclodextrin, and (c) 1% sulfated ß-cyclodextrin. Each peak corresponds to (1) PG, (2) DHS, (3) Pr, and (4) Bz.

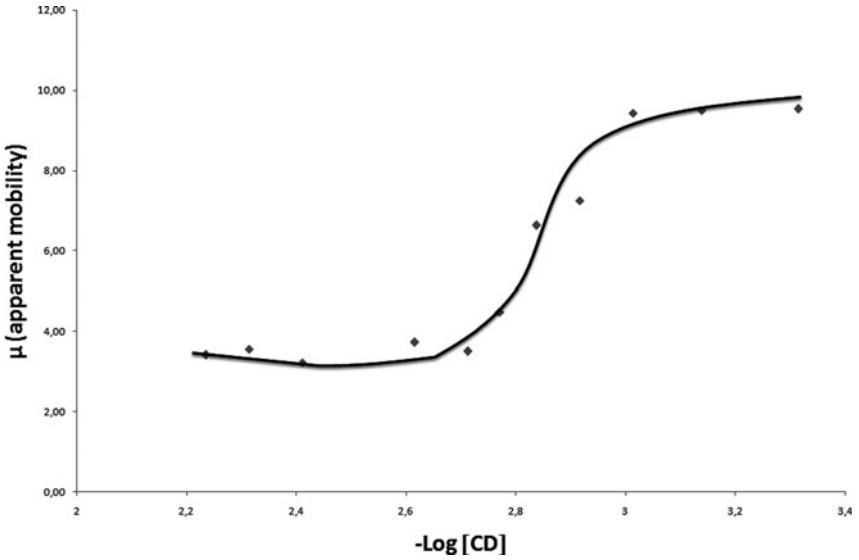

Fig. 3. Plot of apparent mobility of Procaine ($\mu = 10^{-5}\,m^2\,V^{-1}\,S^{-1}$) versus –Log [CD].

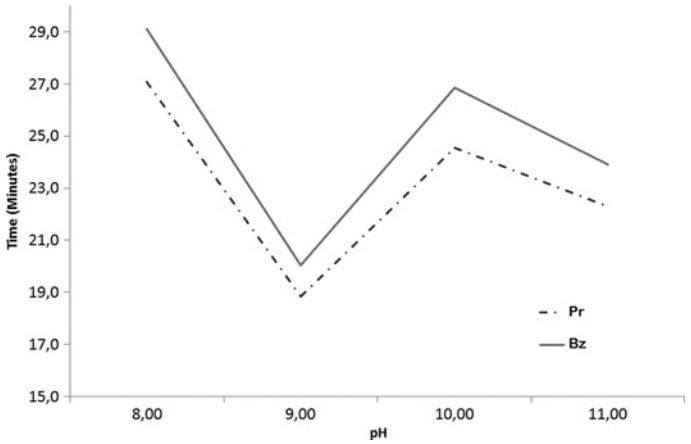

Fig. 4. Plot of migration times of procaine and benzathine versus different pH buffers.

the report of Li et al.[16] We demonstrated that only procaine interacts with sulfated-β-cyclodextrin, and an estimated binding constant value of 8.51×10^{2} was obtained (Figure 3).

The influence of different concentrations of sulfated-β-cyclodextrin (0.5–1.5% w/v) was investigated. At a concentration value of 0.5% w/v, no resolution was obtained between Pr and DHS. Although at 1% and 1.5% w/v the resolution values were quite similar, the pronounced increment in the current intensity at the higher concentration made the system unstable. Therefore, 1% of sulfated-β-cyclodextrin was chosen as the final concentration.

Effect of Buffer pH

Borate buffer was chosen based on a previous report[17] that showed that complexation between monosaccharides and oligosaccharides with borate increases UV absorbance. In order to evaluate the influence of pH in resolution, different BGE with a pH in a range from 8 to 11 were assayed. Resolution between Pr and Bz varies in a range from 5.3 to 7.8, wherea the resolution between DHS an Pr remains without major changes. As is shown in Figure 4, the best resolution with the shorter migration times was obtained at pH 9. As a result, this value was selected as the buffer pH for BGE. Resolution was calculated according to USP 31.[18]

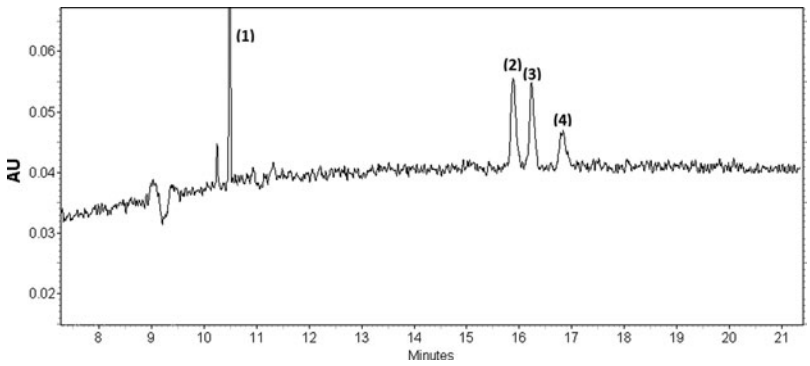

Fig. 5. Electropherogram of a standard solution of of DHS, PGPr, and PGBz at BGE final conditions: (1) PG, (2) DHS, (3) Pr, and (4) Bz.

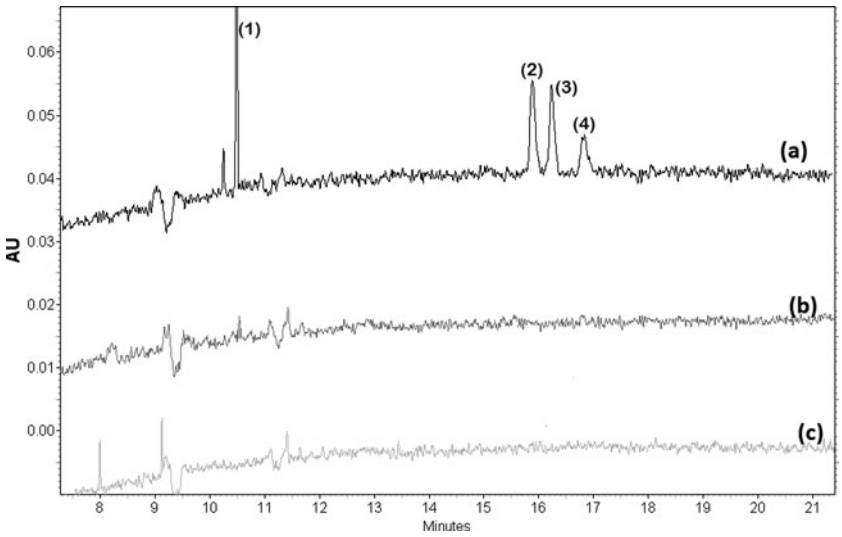

Fig. 6. Electropherogram of (a) standard solution of DHS, PGPr, and PGBz, and (b)–(c) of excipients: blank in methanol and water, respectively. Each peak corresponds to (1) PG, (2) DHS, (3) Pr, and (4) Bz.

Effect of Organic Solvent

The effect of organic modifiers on retention and selectivity has been widely studied over the years. Among different theories, it cannot be denied that the addition of organics modifiers to the BGE with SDS micelles not only does alter parameters related with the micelle size and shape, but also the partition of the analytes between the micelles and the aqueous phase. Tetrahydrofuran, acetonitrile, and methanol were tested as organic modifiers at 5% v/v concentration. As it was expected, the addition of the organic modifier enlarges the elution window in all three cases; however, no significant increase in resolution was observed, compared with the increment in the migration times. The increment on the migration times was less pronounced for acetonitrile, which is why different concentrations of this solvent were tested (2.5–5.0–10% v/v). No significant improvement in the resolution was obtained with respect to the BGE without organic modifier (Figure 5); thus, the absence of organic modifier in the BGE was preferred.

Effect of Instrumental Parameters on the Resolution

Different instrumental parameters were investigated to evaluate their impact in the resolution. Voltage was tested from 15 to 25 kV, and temperature was also tested in a range from 30 to 40°C. When 20 kV was tested, the current increased to an undesirable value, and, thus, a slight reduction in the voltage was applied to avoid high current values. Temperature was a key factor in the resolution, as higher temperatures improve the shape of the peaks increasing resolution; however, with temperatures over 37°C the system became unstable. A temperature of 35°C and a voltage of 18 kV were selected for further analysis as a compromise between resolution and migration times.

Finally, after an exhaustive optimization, the chosen system consisted of 50 mM SDS, 1% sulfated-ß-cyclodextrin sulfated, and 10 mM pH 9.0 borate buffer without organic modifier. Capillary temperature was kept at 35°C, and the electrophoretic system was operated under positive polarity at a constant voltage of 18 kV (Figure 5).

Method Validation

Once the method was optimized, it was exhaustively validated. Validation method was accomplished according to ICH

Table 2. Validation parameters

Linearity		
	Range (μg/mL)	
DHS	30.0–330.0	$y = 405.9\ X - 12904\ R^2 = 0.990$
PGP	15.0–150.0	$y = 1424\ X - 2482\ R^2 = 0.993$
PGB	15.0–150.0	$y = 509.4\ X - 8272\ R^2 = 0.992$

LOD and LOQ		
	LOD (μg/mL)	LOQ (μg/mL)
DHS	2.3	7.8
PGP	1.2	3.9
PGB	3.4	12.8

Precision				
	Migration times [RSD (%)]		Peak area [RSD (%)]	
	Intraday (n = 6)	Interday (n = 18)	Intraday (n = 6)	Interday (n = 18)
DHS	0.7	1.3	1.8	2.0
PGP	0.9	1.7	2.0	1.9
PGB	0.6	1.1	2.0	2.0

Accuracy			
	80%*	100%*	120%*
DHS**	96.1 (0.8)	98.6 (1.4)	98.2 (1.5)
PGP**	96.3 (1.4)	99.1 (1.8)	96.7 (1.4)
PGB**	97.2 (1.6)	98.8 (1.5)	100.3 (1.6)

*respect to the labeled value.
**RSD values between brackets corresponding to $n = 3$.

guidelines.[19] Parameters such as specificity, linearity, LOD and LOQ, precision, accuracy, and robustness were performed.

Specificity was tested by comparing electropherograms of a mixed standard solution of the three analytes (Figure 6a) with an excipients blank prepared exactly as the sample solutions. No interference was observed (Figure 6b–c). Linearity for each analyte was assayed in an interval from 30 to 330 µg/mL for DHS, and from 15 to 150 µg/mL for PGPr and PGBz (five points each injected by triplicate). LOD and LOQ were determined as three and

Table 3. Robustness results DHS

	Migration time			N			R			Found label		
Injection Time	2	3	4	2	3	4	2	3	4	2	3	4
Mean Value	15.94	15.86	15.96	110691	109826	110564	2.77	2.77	2.76	19.3	19.1	19.4
SD	0.05	0.12	0.19	335.51	488.07	532.36	0.01	0.02	0.01	0.06	0.06	0.10
Voltage	16	18	20	16	18	20	16	18	20	16	18	20
Mean Value	16.28	15.86	15.56	108861	109566	111040	2.80	2.76	2.70	19.2	19.1	19.4
SD	0.05	0.12	0.21	741.05	265.17	608.32	0.01	0.01	0.02	0.14	0.06	0.06
Temperature	33	35	37	33	35	37	33	35	37	33	35	37
Mean Value	16.22	15.86	15.91	110546	109826	111549	2.65	2.77	2.81	19.2	19.1	19.2
SD	0.09	0.12	0.07	1257.26	488.07	1139.63	0.04	0.02	0.02	0.06	0.06	0.23
pH Buffer	8.5	9.0	9.5	8.5	9.0	9.5	8.5	9.0	9.5	8.5	9.0	9.5
Mean Value	16.16	15.86	15.63	110712	109826	110691	2.71	2.77	2.77	19.2	19.1	19.2
SD	0.10	0.12	0.01	1124.63	488.07	277.07	0.01	0.02	0.01	0.14	0.06	0.05

Table 4. Robustness results PGPr

	Migration time			N			R			Found label		
Injection Time	2	3	4	2	3	4	2	3	4	2	3	4
Mean Value	16.41	16.39	16.38	98789	98765	99137	6.50	6.46	6.47	9149405	9139338	9159924
SD	0.04	0.04	0.04	590.35	240.50	759.84	0.09	0.06	0.04	45240.42	92157.61	39739.90
Voltage	16	18	20	16	18	20	16	18	20	16	18	20
Mean Value	16.57	16.39	16.06	98770	98765	99022	6.47	6.47	6.17	9138236	9139338	9155682
SD	0.05	0.04	0.06	558.61	340.12	568.59	0.06	0.08	0.61	64284.5	92157.6	42611.1
Temperature	33	35	37	33	35	37	33	35	37	33	35	37
Mean Value	16.21	16.39	16.42	98403	98765	98574	6.45	6.46	6.49	9141894	9139338	9159991
SD	0.08	0.04	0.02	171.40	240.50	702.54	0.04	0.06	0.04	109179.60	92157.61	98112.66
pH Buffer	8.5	9.0	9.5	8.5	9.0	9.5	8.5	9.0	9.5	8.5	9.0	9.5
Mean Value	16.71	16.39	16.22	98602	98765	98856	6.39	6.46	6.53	9146044	9139338	9158641
SD	0.05	0.04	0.05	407.13	240.50	588.13	0.02	0.06	0.04	14743.41	92157.61	137698.48

Table 5. Robustness results PGBz

	Migration time			N			Found label		
Injection Time	2	3	4	2	3	4	2	3	4
Mean Value	16.97	16.94	16.91	45820	44946	44243	10770913	10790573	10750318
SD	0.13	0.09	0.03	1437.75	944.22	423.39	39271.51	102119.27	69775.90
Voltage	16	18	20	16	18	20	16	18	20
Mean Value	17.29	16.94	16.70	45142	44958	43963	10726459	10790573	10802505
SD	0.05	0.09	0.05	905.80	1335.02	546.01	39899.91	102119.27	116926.49
Temperature	33	35	37	33	35	37	33	35	37
Mean Value	17.20	16.94	16.83	44662	44946	44031	10794414	10790573	10826766
SD	0.08	0.09	0.06	890.54	944.22	45.00	109573.11	102119.27	145815.98
pH Buffer	8.5	9.0	9.5	8.5	9.0	9.5	8.5	9.0	9.5
Mean Value	17.11	16.94	16.70	44902	44946	44900	10763641	10790573	10788757
SD	0.09	0.09	0.05	1617.43	944.22	948.52	113614.58	102119.27	13075.20

Table 6. Application of the MEEKC system to the analysis of a veterinary formulation

		Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
PGPr	Found label (UI/100 mL)	9139338	9521513	10785356	10427568	9983987
	RSD*	1.0	1.2	0.5	0.8	1.5
	Percentage **	91.4	95.2	107.9	104.3	99.8
PGBz	Found label (UI/100 mL)	10790753	9485340	10123862	10689234	9065123
	RSD*	0.9	1.4	1.4	0.8	0.9
	Percentage**	107.1	94.9	101.2	106.9	90.7
DHS	Found label (% w/v)	19.1	20.2	21.4	19.4	21.7
	RSD*	0.3	0.6	0.5	0.5	0.9
	RSD* Percentage**	95.5	101.0	107.0	97.0	98.6

*$n = 3$.
**Respect to the labeled claim.

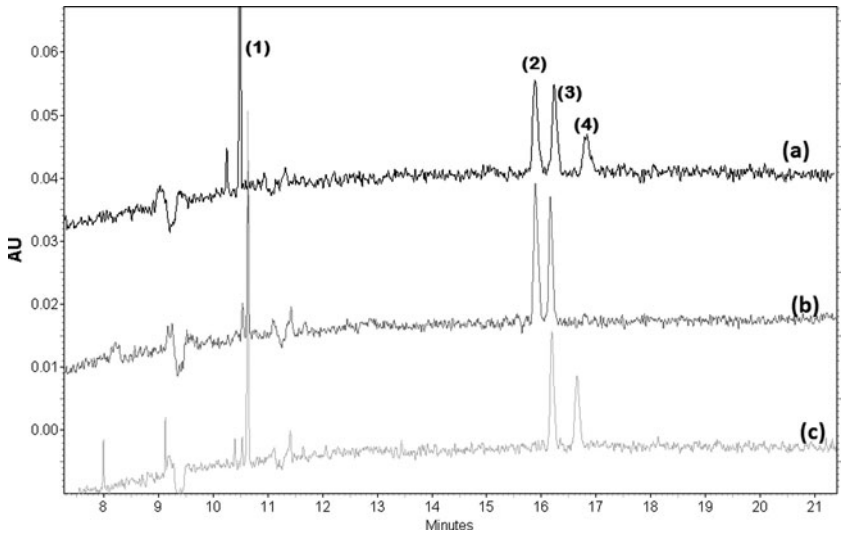

Fig. 7 Electropherogram of (a) Standard solution of DHS, PGPr, and PGBz; (b) sample prepared in methanol; and (c) sample prepared in water. Each peak corresponds to (1) PG, (2) DHS, (3) Pr, and (4) Bz.

ten times the ratio signal-to-noise, respectively (3 S/N and 10 S/N).

Precision was evaluated for intraday ($n = 6$) and interday ($n = 18$) assays and it was expressed as % of RSD for peak areas. Accuracy was tested from recovery studies. Placebo samples prepared with all the excipients present in the formulation were spiked with each analyte at concentration levels of 80%, 100%, and 120% of the nominal value. Three replicates of each level were assayed. Results are shown in Table 2.

Robustness was statistically analyzed after evaluating the effect of varying different electrophoretic parameters: time injection, voltage, and temperature. The method capacity to remain unaffected was studied for standard solutions and samples, and parameters selected for two levels of variation (upper and lower) were: time injection ($3\,s \pm 1$), voltage ($18\,kV \pm 2$), temperature ($35 \pm 2°C$), and buffer pH (9.00 ± 0.5). The effects of variations on the electrophoretic parameters were statistically evaluated applying a t-student test according to international guidelines[18,19] using migration time, mean theoretical plates, resolution, and sample concentration. The data obtained in these experiments did not show a significant difference of means (p-value >0.05) (Table 3, 4, and 5).

Application

To demonstrate its suitability to quality control, the developed electrophoretic system was applied to the analysis of five batch of a commercially available suspension (Procilina Strepto, Proser S.A.) containing 20% w/v of dihydrostreptomycin sulfate and 10.000.000 UI/100 mL of penicillin G benzathine and penicillin G procaine for veterinary use. Preparation of standards and samples preparation is shown in – the Effect of Cyclodextrin Type and Concentration section. The obtained results ranged between 90% and 115% of the labelled claim (Table 6 and Figure 7).

Conclusions

The developed CD-MEKC system was proven suitable for the simultaneous determination of penicillin G procaine, penicillin

G benzathine, and dihydrostreptomycin sulfate. The method was fully validated in terms of specificity, linearity, LOD, LOQ, accuracy, precision, stability, and robustness. This method is suitable for the routine quality control due to satisfactory results in terms of precision and accuracy, according to the requirements of the regulatory agencies.

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