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Assessment of MEKC suitability for residue drug monitoring on pharmaceutical manufacturing equipment

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Abstract

The suitability of micellar electrokinetic chromatography for the simultaneous trace determination of several compounds (sulfamethoxazole, trimethoprim, sulfanilic acid, sulfanilamide, 3,4,5-trimethoxybenzoic acid and nonoxynol-9) was assessed. The mixture was separated within 14 min at an applied voltage of 22 kV by using 30 mM phosphate electrolyte, containing 10 mM SDS, adjusted to pH 7.8. Under optimized separation conditions acceptable levels of linearity, precision and accuracy were obtained for all compounds. The method could be used as part of a cleaning validation study when assaying trace levels of co-trimoxazole drug, some of its decomposition products and detergent in the swab samples collected from pharmaceutical manufacturing equipment, after cleaning.
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Keywords: MEKC; Cleaning validation; Co-trimoxazole; Antibacterial drug; Sulfamethoxazole; Trimethoprim; Sulfanilic acid; Sulfanilamide; 3,4,5-Trimethoxybenzoic acid; Nonoxynol-9

1. Introduction

This article presents the development and validation of a micellar electrokinetic chromatography (MEKC) method for the simultaneous determination at trace levels of compounds likely to remain on pharmaceutical manufacturing equipment after cleaning. The cleaning validation sample studied potentially contains traces of active pharmaceutical ingredients (APIs) of co-trimoxazole drug product, some of their decomposition products and detergent components.

Co-trimoxazole is a bacteriostatic antibiotic combination of sulfamethoxazole (SMZ) and trimethoprim (TMP) in a 5:1 (w/w) ratio, formulated as injections, oral suspensions and tablets under different brand names. SMZ is a sulfonamide, having a structure analogous of *p*-aminobenzoic acid and acts as a competitive inhibitor of dihydropteroate synthetase. TMP acts by interfering with the action of bacterial dihydrofolate reductase, inhibiting the synthesis of tetrahydrofolic acid. When TMP is used in combination with SMZ, co-trimoxazole exhibits a synergistic antibacterial effect by inhibiting successive steps in the folic acid metabolism of bacteria. Co-trimoxazole is used in the treatment of sinusitis, pneumonia, chronic bronchitis, renal and urinary tract infections, gastrointestinal tract infections, skin and wound infections, septicaemias and other infections caused by sensitive organisms [1].

Typically, for cleaning validation, assay methods are developed for APIs or for the most toxic, potent or difficult to remove compound of a drug [2]. Simultaneous HPLC determinations of SMZ and TMP in various matrices have been described in a number of publications [3–8]. A pharmacopoeial method for the simultaneous determination of SMZ and TMP by HPLC is also available [9].

It is recommendable to also test for the presence of potential toxic decomposition products. TMP was determined through HPLC in the presence of its decomposition products [10]. The separation of sulfonamides in the presence of their decomposition products was not possible through HPLC as the elution of sulfanilamide (SAM) and sulfanilic acid (SAA) takes place at or near the void volume, causing problems with their identification and quantification [11].

Generally, the pharmaceutical manufacturing equipment is cleaned with aqueous solutions of detergents at pre-defined

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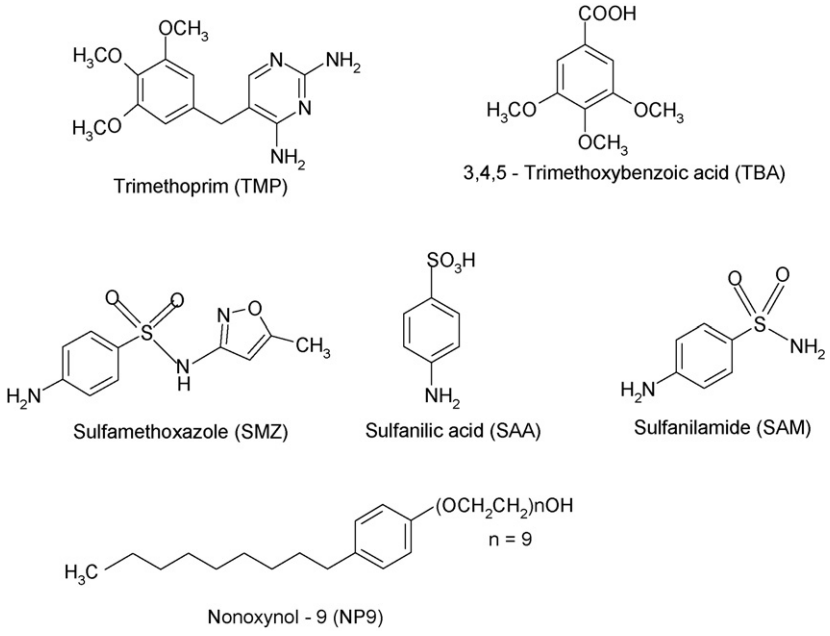

Fig. 1. Chemical structures of trimethoprim, 3,4,5-trimethoxybenzoic acid, sulfamethoxazole, sulfanilic acid, sulfanilamide and nonoxynol-9.

concentrations and temperatures. The detergents may constitute themselves a source of product contamination and therefore regulatory agencies such as Food and Drug Administration [12] require the availability of a validated method capable of detecting traces of detergent after the manufacturing equipment was cleaned and thoroughly rinsed. In practice, all these requirements may result in extensive sample preparation followed by analysis via several different techniques.

Nonoxynol-9 (NP9) is the wetting agent present in the composition of the detergent selected to clean co-trimoxazole drug. NP9 consists of mixtures of oligomers of polyethoxylated nonylphenol, a non-ionic surfactant miscible with water. The US Pharmacopoeia describes an HPLC method for assaying NP9. However, according to [13] the USP method does not provide enough sensitivity for determining NP9 in the lower concentration range required for cleaning validation. An HPLC method [13] has been validated for the determination of residual NP9 in cleaning validation samples collected from stainless steel, polytetrafluoroethylene, and acrylic substrates.

Capillary electrophoresis has the advantage of separating analytes differing in their charge-to-radius ratio. It is also sensitive enough to allow the quantification at trace levels of drug-related impurities and residues found in cleaning validation samples. A single assay would offer savings in time, effort and convenience [14]. Capillary zone electrophoresis (CZE) separations have been possible for SMZ and TMP due to their chemical nature. SMZ is a weak acid (pKa = 5.6) and TMP is a weak base (pKa = 6.6). SMZ and TMP were simultaneously determined through CZE with UV detection [15–17] in fused silica capillaries. A polyamine coated capillary was used to separate TMP and some of its decomposition products including 3,4,5-trimethoxybenzoic acid (TBA) [18]. A microfluidic capillary electrophoresis method combined with flow injection analysis was developed for the on-line concentration and separation of SMZ and TMP [19]. CZE with amperometric detection was used to determine TMP and several sulfonamides [20]. MEKC was used in the determination of several sulfonamides [21], sulfonamides and TMP [22,23] and also in the determination of SMZ, TMP and other drugs in human plasma without sample pretreatment [24]. The migration behaviour of thirteen sulfonamides was studied by both CZE and MEKC [25].

At present there is no analytical method available, capable to determine in a single run the APIs of co-trimoxazole, its decomposition products and traces of detergent, potentially present in the cleaning validation sample. The chemical structures of the compounds for which the present method is intended to are shown in Fig. 1.

2. Materials and methods

2.1. Equipment

CE separations were performed on a Hewlett Packard 3D CE instrument with HP ChemStation software. Uncoated silica capillaries, from Macherey-Nagel were obtained from separations (Johannesburg, South Africa). The typical capillary dimensions were 50 μm i.d., 375 μm o.d., 58 cm total length (L) and 50 cm effective length (L_{det}) from the inlet to the detector window. On-line UV detection was performed at 210 nm with a diode array detector. The capillary was maintained at 25 °C with forced air circulating in the capillary oven. The high voltage power supply was set to 22 kV (normal polarity equivalent to a field strength of 379.3 V/cm resulting in a typical current of 50 μA). The detection window was at the cathodic side. Injections were made at the anodic side of the capillary in the hydrodynamic mode (0.75 psi ≈50 mbar) for 4 s of sample followed by 2 s of electrolyte.

Before the first use, the capillary was conditioned with freshly prepared 0.1 M NaOH for 6 h at 25 °C in order to regen-

erate the silanol groups on the capillary surface. At the start of a working day the capillary was regenerated by rinsing with 0.1 M NaOH (15 min), water (5 min) and electrolyte solution (15 min). Between-run washings were performed with water (1 min), 0.1 M NaOH (1 min), water (1 min) and electrolyte solution (2 min). At the end of every day the capillary was rinsed with water (5 min), 0.1 M NaOH (15 min), water (5 min) and methanol (5 min). Air was flushed for 15 min prior to capillary storage.

For pH measurements a Mettler Toledo MPC 227 pH-meter with a precision of 0.01 pH units was used. Daily calibrations of the pH sensor were performed with buffers of the following pH values: 4.01, 7.00 and 9.21.

2.2. Chemicals and reagents

All chemicals were of analytical grade. Sodium hydroxide, di-sodium hydrogen phosphate, sodium di-hydrogen phosphate and sodium dodecyl sulfate (SDS) were purchased from Merck (Wadeville, South Africa). HPLC grade methanol was purchased from Lab-Scan (Dublin, Ireland). Nonoxynol-9 (Sigma), sulfanilic acid (Fluka) and 99.96% sulfanilamide (Fluka) were purchased from Sigma–Aldrich (Kempton Park, South Africa). 99.90% Sulfamethoxazole (4-amino-*N*-(5-methyl-3-isoxazolyl) benzenesulfonamide), 99.94% trimethoprim (5-[(3,4,5-trimethoxyphenyl) methyl]-2,4-pyrimidinediamine) and 98.60% 3,4,5-trimethoxybenzoic acid certified standards were supplied by Roche Diagnostics (Mannheim, Germany). The calibration buffers (pH 4.01, 7.00 and 9.21) were purchased from Microsep (Johannesburg, South Africa).

Solutions were prepared with deionized water, produced by a MilliQ® water purification system from Milipore (Bedford, MA, US). Swab samples were prepared with Bemcott M_3 wipers purchased from Asahi Chemical Industry (Tokyo, Japan).

2.3. Preparation of background electrolyte and solutions for standards and samples

The background electrolyte (BGE) was prepared by mixing different proportions of di-sodium hydrogen phosphate and sodium di-hydrogen phosphate solutions of equal molarities to the desired pH value. Various quantities of SDS were dissolved in the electrolyte already adjusted to the final pH in order to obtain the desired SDS concentrations.

Independent stock solutions were prepared in 25 ml volumetric flasks by dissolving SMZ (248.9 mg), TMP (76.3 mg) and TBA (126.1 mg) in 15 ml methanol. All three volumetric flasks were then brought to volume with MilliQ® water. Other independent stock solutions were prepared in 25 ml volumetric flasks for NP9 (661.5 mg), SAA (176.9 mg) and SAM (301.6 mg) by dissolving in MilliQ® water.

Intermediate independent stock solutions were prepared at eight concentration levels (between 5% up to 200% of the nominal concentration level) by further diluting aliquotes of the initially prepared stock solutions, with a mixture of methanol–MilliQ® water 50:50 (v/v), in 25 ml volumetric flasks.

Working standard solutions were prepared at eight concentration levels by transferring 0.5 ml of each of the intermediate stock solutions into a 10 ml volumetric flasks. Each of the eight volumetric flasks prepared for each concentration level was brought to 10 ml volume with 3 mM NaOH. The final injection solution corresponding to the nominal concentration level (100%) contains 39.82 μg/ml SMZ, 12.21 μg/ml TMP, 20.18 μg/ml TBA, 28.30 μg/ml SAA, 48.26 μg/ml SAM and 105.84 μg/ml NP9.

Swab samples were prepared for each concentration level studied in order to assess the method accuracy by recovery. Three pairs of swabs were placed into 15 ml test tubes. The swabs were spiked with 0.2 ml of the intermediate stock solution corresponding to each analyte. The swabs were allowed to dry and then were extracted in 10 ml of 3 mM NaOH.

2.4. Establishing limits for the contamination and cross-contamination level permitted on cleaned equipment

For products with a defined therapeutic dose, the maximum permitted quantity of API residue (R) allowed on the equipment surface can be calculated by using the 0.1% dose limit criterion. The 0.1% dose limit criterion is justified by the principle that, generally, an API at a concentration of 1/1000 of its lowest therapeutic dose will not produce any adverse effects to one's health. This criterion as explained by [26] accounts for the maximum daily intake of a following product and for the batch size of the product that will be manufactured next with the same equipment and for the total surface area of the equipment chain in direct contact with the product (Eq. (1)):

$$R\,(\mu g/cm^2) = \frac{DS}{IF} \times \frac{1}{A} \quad (1)$$

The 10 ppm criterion is another method [26] of establishing limits for the contamination permitted on manufacturing equipment, after cleaning. According to this criterion no more than 10 ppm of a product is allowed to migrate into the next manufactured product (Eq. (2)):

$$R\,(\mu g/cm^2) = 10 \times 10^9 \times S \times \frac{1}{A} \quad (2)$$

where R = maximum residue of API permitted after cleaning, allowed on the surface of manufacturing equipment, in μg/cm^2; it is assumed that the total amount of residue is distributed homogenously into the following product; D = lowest daily therapeutic dose of the contaminant, in mg; S = smallest batch size for any subsequent product, in kg; I = maximum daily intake of any product manufactured with the same equipment, in mg; F = safety factor (can vary from 10 to 10,000 depending on the route of product administration e.g., topical, oral or injectable preparations). More stringent values down to 1/100 000th of the lowest therapeutic dose may be used for research, investigational products and products such as hormones and antibiotics that can still cause an allergenic effect even in very small doses; A = total surface area of equipment in direct contact with the product, in cm^2, calculated based on the assumption that all

the product comes into contact with all the parts of the manufacturing equipment; 10^9 is a transformation factor from kg to μg.

So far, the problem of cross-contamination addresses limits established for drug related contaminants. Cross-contamination refers to the contamination of a product by a previously manufactured product, whereas contamination refers to any chemical, microbiological or particulate contaminant likely to remain on the equipment surface after cleaning. Therefore all detergent components, including NP9 are referred to as equipment and product contaminant.

For detergent, an acceptable daily intake (ADI) will be defined based on toxicological limits [27]. The maximum allowable residue (MAR) per process step or equipment can be calculated with the following equations:

$$\text{ADI} = \text{NOAEL} \times \text{AAW} \times F \tag{3}$$

$$\text{NOAEL} = \text{LD}_{50} \times \text{EF} \tag{4}$$

$$\text{MAR} = \text{ADI} \times \frac{S}{I} \tag{5}$$

where ADI = acceptable daily intake, in mg; NOAEL = no observed adverse effect level, in mg/(kg day); AAW = average adult weight, in kg (=70 kg); EF = empirical factor derived from animal model developed by [27] (=0.0005); LD_{50} = lethal dose 50% kill, in mg/kg; MAR = maximum allowable residue in μg/cm^2—will be calculated taking into account all equipment surface areas; *F*, *S*, and *I* have the same significance as for Eq. (1).

2.5. Determination of recovery rate of contaminants from stainless steel and plexiglass surfaces

Recovery rate studies are performed in order to determine to what extent the residue may be retrieved from the production equipment with the sampling procedure tested. SMZ, TMP and NP9 were independently spiked on 316 stainless steel and plexiglass coupons (10 cm × 10 cm) at levels corresponding to the limit of contamination (LOC) previously calculated. The spiked coupons were allowed to dry (ca. 2 h) at room temperature. Since the swabbing is an operator dependant technique, each analyte was recovered five times from coupons in order to obtain a representative mean of the recovery rate. The swab sampling simulation was performed by one operator.

A pair of tweezers was used to perform the swabbing. Various solvents (e.g., methanol, ethanol, MilliQ® water) were considered for swabbing. The first swab was wetted with 0.2 ml of swabbing solvent. The coupon was swabbed horizontally with one side of the swab and vertically with the other side. The procedure was repeated two more times with two more wetted swabs. All three swabs were collected into the same test tube. Before injecting the sample, appropriate dilutions were performed in order to bring the concentration of the sample within the validated range of the analytical method.

3. Results and discussion

3.1. MEKC method development and optimization

For the present MEKC separation the electrophoretic mobilities of the test solutes were calculated in order to evaluate the effects of electrolyte pH, voltage, the concentration of surfactant and the ionic strength on the overall separation. Each parameter was optimized individually. The migration time of the solute, t_m, and the migration time of the neutral marker, t_{eo}, were measured from the electropherograms. Methanol was used as neutral marker. Other factors such as the influence of organic modifier, temperature and injection time are not discussed in this article.

To ensure that the experiments produce reproducible results all runs were carried out at least in triplicate.

3.1.1. pH influence

The pH of the BGE determines the degree of ionization of individual solutes and their net charge in solution. The pH influence on the behaviour of solutes was studied in the range from 6.6 up to 8.2 in increments of 0.2 pH units. Initial separation conditions were 25 mM phosphate electrolyte, 20 mM SDS, 20 kV and 25 °C.

SAM has a p*K*a of 10.43 and therefore at any pH below this value is neutral and elutes close to the electroosmotic flow (EOF). SMZ (not all p*K*a's found in literature) is an ampholyte with possibly several ionization configurations due to its three nitrogen groups and one sulphoxy group. TBA is a monocarboxylic acid, negatively ionized in the pH range studied. As the pH was increased, the selectivity factor (α) between SMZ and TBA has decreased. Above 7.2 pH units α has a constant value of 1.02 that allows for the quantitative determination of the two analytes. Baseline resolution between the SMZ and TBA peaks was constantly achieved for the entire range of pH investigated.

SAA is a small anion and the pH variation could influence more readily its charge/mass ratio. For SAA a slight decrease in the migration time was observed with the increase of pH. It is believed that in the pH range studied SAA and SMZ are dissociated and negatively charged. They migrate slowly towards the cathode as a result of their electrophoretic behaviour.

In agreement to the Henderson–Hasselbach equation for weak bases [28], when the pH shifts from acidic to basic, TMP undergoes a variation from neutral to cationic. Fig. 2 shows that up to a pH value of 7.2 TMP could not be separated from NP9 as both being neutral and therefore eluting together within the micellar phase. The complete baseline resolution of TMP and NP9 was obtained at pH 7.8 where more than 90% of the TMP is in its cationic state and mostly distributed in the aqueous phase. The conclusion is that the elution behaviour of the TMP is mainly governed by the CZE mechanism. Because NP9 is a mixture of non-ionic oligomers its migration was not influenced by pH variations.

A final pH of 7.8 was selected for the phosphate electrolyte for further method optimization as it offers good buffering capacity. Fig. 2 presents the influence of the electrolyte pH on the electrophoretic mobilities of the solutes studied. As it can be observed there is very little influence of the pH on the solute

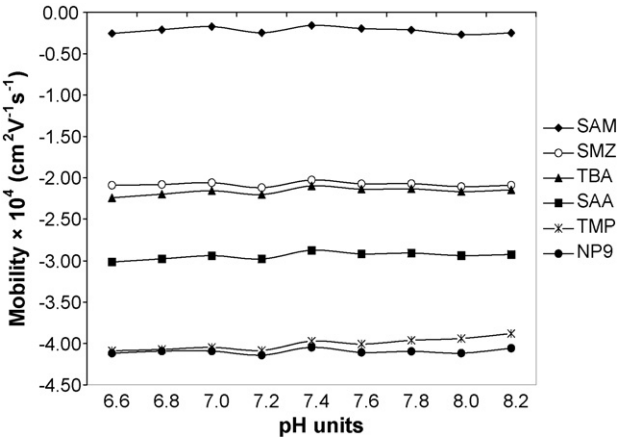

Fig. 2. Electrophoretic mobilities of solutes obtained as a function of pH in the range 6.6–8.2; 25 mM phosphate electrolyte containing 20 mM SDS. Operating conditions: 20 kV; 25 °C.

electrophoretic mobilities. It is to be emphasized that the electrophoretic mobilities given are in all cases pseudoeffective electrophoretic mobilities which are not only determined by the charge/radius ratio but also by the distribution coefficient of solutes between the aqueous and micellar phase and by the volume ratio of these two media.

3.1.2. Voltage influence

Voltage influence was studied in the range between 18 kV up to 28 kV in increments of 2 kV by using the previously found optimum pH value of 7.8 for the phosphate electrolyte. Fast sample analysis, moderate current values of ca. 58 μA and a power consumption of ca. 1.62 W was obtained at 22 kV.

3.1.3. Influence of surfactant concentration

SDS was used as surfactant in order to facilitate the separation of NP9 through MEKC mechanism. The SDS influence was studied for the concentration interval ranging from 10 to 50 mM SDS in increments of 5 mM units. All other parameters were kept constant while varying the SDS concentration. As the SDS concentration increases the NP9 peak becomes very broad due to its non-ionic oligomers starting to separate. At higher SDS concentrations problems related to capillary blockage were encountered. A concentration of 10 mM SDS was considered sufficient in order to allow for the optimum separation of NP9.

3.1.4. Ionic strength influence

The influence of ionic strength was studied on electrolyte solutions with phosphate concentrations varying between 10 and 50 mM. Currents ranging from 24 μA up to 102 μA were obtained. By increasing the ionic strength the buffering capacity increases with benefits for separation. Above 35 mM the Joule effect becomes noticeable through heating and consequently peak broadening, especially towards the end of the separation. However, at low BGE concentrations the TMP and NP9 peaks are overlapping. A concentration of 30 mM phosphate was selected to ensure an optimum ionic strength of the electrolyte. In terms of electrolyte ionic strength this corresponds to a calculated value of 107.7 mM. The electrolyte concentrations of weak acid and base used in the formula $\frac{1}{2}\sum_i c_i z_i^2$ (where c_i is the molar concentration of ion i in solution and z_i is the net charge of ion i in solution) were determined with the help of Henderson–Hasselbalch equation [28].

One disadvantage of using MEKC for cleaning validation is the on-column detection capability of capillary electrophoresis, which limits the sensitivity of the method due to the small optical path length. To increase the method sensitivity, larger injection times were used cautiously so that capillary overloading and peak distortion/tailing phenomena were avoided. An injection time of 4 s of sample followed by 2 s of BGE was selected for further experiments.

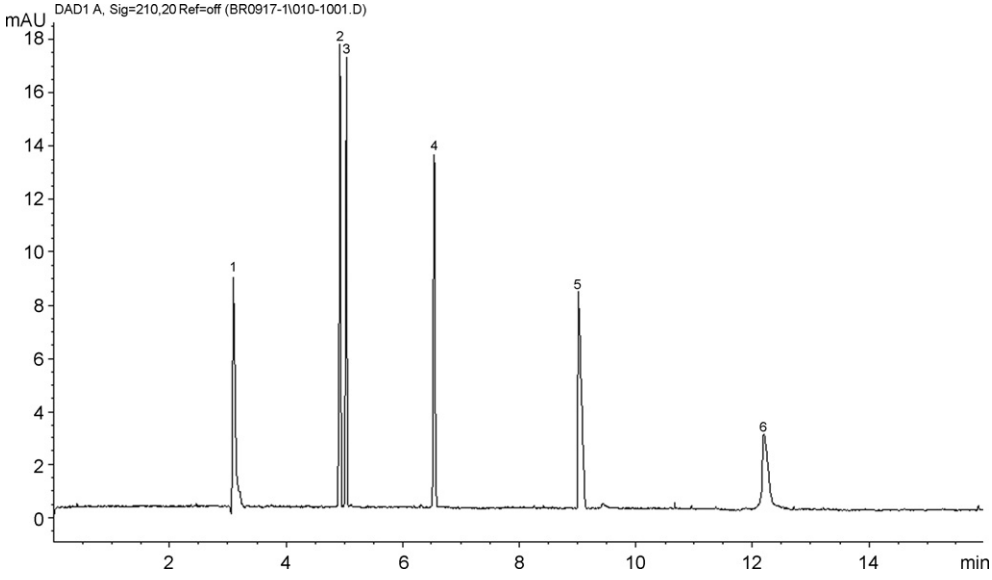

Fig. 3. Electropherogram obtained with the optimized migration conditions (30 mM phosphate electrolyte, adjusted to pH 7.8, containing 10 mM SDS, applied voltage of 22 kV and 25 °C). Injection: 4 s sample followed by 2 s electrolyte. Migration times are 3.09 min for SAM (1), 4.92 min for TBA (2), 5.02 min for SMZ (3), 6.54 min for SAA (4), 9.01 min for TMP (5) and 12.20 min for NP9 (6).

Final separation conditions were established as 30 mM phosphate electrolyte adjusted to pH 7.8 and containing 10 mM SDS, at an applied voltage of 22 kV and 25 °C. With the above separation conditions the six sample components were resolved within 14 min. A typical electropherogram of the six separated analytes is shown in Fig. 3. Each analyte is completely resolved.

The method development and validation were performed on a dedicated capillary over a period of ca. 7 months. During this time the capillary had to be changed once due to blockage. The pH was moved across the range for a number of times during method development. These changes had limited effect with regards to the capillary behaviour and similar results were obtained in all situations. This fact is very important for the highly regulated pharmaceutical environment that requires robust analysis methods and reproducible results over extended time periods.

3.2. MEKC method validation

3.2.1. Method range

The validation parameters assessed for determining the method range were the linearity, precision (by repeatability and reproducibility) and the accuracy (by recovery).

Linearity was studied by injecting standard solutions prepared at eight concentration levels with four replicates per level and evaluating the peak area obtained. A linear regression least square analysis was performed in order to determine the slope, intercept and correlation coefficient of the standard curves. The coefficients of correlation (r) are higher than 0.996 for all the analytes studied. The intercepts of the regression lines are situated for all the analytes within the 95% confidence band of $\pm 10.0\%$ of $\langle Y \rangle_{Ref}$ indicating that there are no constant systematic errors.

The limits of detection (LOD = 3.3 s/S) and quantification (LOQ = 10 s/S) were calculated. 'S' is the slope of the calibration curve and 's' the standard deviation. For the estimation of the standard deviation 's', the standard deviation of the y-intercept was used.

LODs of up to 1 μg/ml and LOQs of up to 2.5 μg/ml were obtained for four of the analytes The LOQ translates to a residue limit of 25 μg/swab. Higher LODs and LOQs were obtained for SAM due to possibly integration problems created by the proximity of the negative peak recorded at the start of the electropherogram. Also, the broad peak obtained for NP9 influences its quantification capabilities. The linearity data are presented in Table 1.

The intra-assay precision of the method, reported as relative standard deviation, was assessed by measuring the repeatability of peak areas and of the migration times obtained for four replicate swab samples at eight concentration levels. The %R.S.D. values obtained per level are presented in Table 2.

The inter-assay precision was assessed based on peak area and migration times obtained for three concentration levels (50, 100, 150%) with six replicate swab samples per level (see Table 3). The assays were carried out over 3 days on the same instrument by one operator ($n = 18$).

The results presented in Tables 2 and 3 indicate a very good repeatability and reproducibility of the migration times. This denotes in turn that the separation conditions are stable between individual runs and from day to day. A higher %R.S.D. of 3.7% was obtained for TMP probably due to the fact that its part distribution between the aqueous and mobile phase, dictated by the working pH, determines a slight variability of its migration times.

An overall %R.S.D. of less than 5% was obtained for the peak area of all analytes, except NP9. These values demonstrate

Table 1
Parameters of linearity for co-trimoxazole mixture compounds

Analyte	Concentration range (μg/ml)	y-Intercept	Slope	r^2	LOD (μg/ml)	LOQ (μg/ml)
SAM	2.41–96.51	−2.043E−01	5.367E−01	0.998	1.423	4.312
SMZ	1.99–79.65	−4.567E−01	6.841E−01	0.999	0.753	2.283
TBA	1.01–40.35	−9.314E−02	1.201E+00	0.999	0.431	1.306
SAA	1.42–56.60	−7.177E−02	8.348E−01	0.998	0.825	2.501
TMP	0.61–24.42	1.135E−01	2.745E+00	0.997	0.394	1.193
NP9	5.29–211.68	1.559E+00	3.184E−01	0.993	5.358	16.237

Table 2
Intra-assay precision (1 day) for co-trimoxazole mixture compounds

Level	Peak area (%R.S.D.)						Migration time (%R.S.D.)					
	SAM	SMZ	TBA	SAA	TMP	NP9	SAM	SMZ	TBA	SAA	TMP	NP9
5%	3.2	4.9	5.5	3.0	6.8	–	0.2	0.6	0.4	0.5	2.3	–
10%	5.6	3.8	3.7	2.9	6.6	8.4	0.6	0.9	0.9	1.2	3.3	1.7
25%	3.1	3.1	3.0	1.6	3.9	6.8	0.4	0.6	0.5	0.7	1.5	1.3
50%	2.5	1.1	1.4	1.5	3.9	3.3	0.3	0.4	0.3	0.3	1.9	0.8
75%	3.0	0.8	0.8	1.8	3.9	1.4	0.1	0.2	0.2	0.3	2.8	0.7
100%	2.5	1.2	1.6	1.4	2.5	1.3	0.4	0.6	0.6	0.8	1.6	1.1
150%	1.7	0.6	0.8	0.9	1.3	1.6	0.1	0.2	0.2	0.2	1.4	0.5
200%	2.7	0.5	0.6	0.8	1.2	4.1	0.1	0.1	0.1	0.1	1.9	0.3
Overall %R.S.D.	3.2	2.5	2.7	1.9	4.2	4.6	0.3	0.5	0.5	0.6	2.2	1.0

Table 3
Inter-assay precision for co-trimoxazole mixture compounds (3 days, one instrument, one operator)

Level	Peak area (%R.S.D.)						Migration time (%R.S.D.)					
	SAM	SMZ	TBA	SAA	TMP	NP9	SAM	SMZ	TBA	SAA	TMP	NP9
50 %	4.2	2.6	3.0	3.1	4.8	5.7	0.7	1.0	1.0	1.4	1.4	3.1
100 %	4.3	4.3	4.4	4.7	3.7	5.6	0.7	1.0	1.0	1.4	3.1	2.4
150 %	3.9	3.3	3.3	3.5	4.5	5.8	0.6	0.6	0.6	0.7	4.8	0.8
Overall %R.S.D. ($n=54$)	4.1	3.5	3.6	3.8	4.3	5.7	0.8	1.0	1.0	1.4	3.7	2.6

Table 4
Accuracy by recovery for co-trimozazole mixture compounds

Concentration level	Recovery (%) ($n=3$)					
	SAM	SMZ	TBA	SAA	TMP	NP9
10%	138.0	113.2	106.7	104.2	105.5	83.6
25%	96.3	89.9	92.3	89.5	104.8	49.4
50%	107.4	101.9	103.0	102.0	96.0	58.0
75%	98.6	98.4	100.7	95.7	92.9	60.3
100%	93.8	99.1	103.3	103.7	95.9	82.6
150%	96.6	98.7	99.9	97.4	92.2	82.0
200%	95.4	97.0	98.6	95.9	90.7	87.0
Average	103.7	99.7	100.64	98.3	96.9	71.8

the suitability of the developed method for cleaning validation.

The method accuracy was determined on spiked and dried swabs that where then extracted in 3 mM NaOH. The accuracy of the procedure was assessed by comparing the analyte amount recovered from swabs versus the known amount in the standard injection solutions, at seven concentration levels, with three replicates ($n=3$) for each concentration level investigated. All analytes were recovered with percentages higher than 95%, this fact indicating that there is no retention of the analyte by the swab material. The only exception was NP9 whose recovery from swab recorded a level of less than 75% (Table 4). The higher recovery rates obtained for the 10% level should not be of concern. From a cleaning validation standpoint was more important to obtain reliable results at the LOC.

3.2.2. Limits for the contamination and cross-contamination level permitted on cleaned equipment

The maximum levels of API residue permitted after cleaning were obtained by using the 0.1% dose limit criterion (Eq. (1)). R values of 4.28 μg SMZ/cm^2 and of 0.86 μg TMP/cm^2 were obtained. When the 10 ppm criterion was applied (Eq. (2)), a limit of 85.70 μg/cm^2 was obtained for both SMZ and TMP, which is much higher than the limits calculated with the 0.1% dose limit criterion. The lowest calculated values were selected as limit of contamination (LOC) for this study.

NP9 was selected as a tracer for assessing the level of detergent residue remaining after cleaning. This decision was made due to its low percentage in the detergent composition, and due to limited detection capabilities of other detergent components. It is also believed that NP9 is one of the least rinsable detergent components and therefore likely to remain on equipment after cleaning.

The maximum acceptable level of detergent, calculated by using the Eqs. (3)–(5) was 19.65 μg NP9/cm^2. The smallest manufactured batch size ($S=900$ L) of co-trimoxazole syrup and the maximum daily intake ($I=20$ ml) of syrup were used in calculations as well as an LD_{50} (NP9) = 1310 mg/kg oral, rat. The manufacturing equipment consisting of a manufacturing tank, mixer, filtration device and holding vessels had a calculated surface area of 105,019 cm^2.

3.2.3. Results for the recovery rate of contaminants from stainless steel and plexiglass surfaces

Several solutions were tested for the recovery of the analytes from coupons made of the same material as the manufacturing equipment. A mixture of methanol–MilliQ® water 70:30

Table 5
Recovery of SMZ, TMP and NP9 from stainless steel and plexiglass coupons

	Analyte type spiked on stainless steel coupons			Analyte type spiked on plexiglass coupons		
	SMZ	TMP	NP9	SMZ	TMP	NP9
Quantity of analyte spiked on coupons (μg/100 cm^2)	428.1	85.5	1971.3	428.1	85.5	1971.3
Quantity of analyte recovered from coupons (μg/100 cm^2)	413.1	81.6	1944.6	413.9	77.5	1832.0
	402.0	76.8	1840.4	405.5	80.4	1778.0
	414.2	82.9	1809.8	409.2	82.7	1869.2
	413.7	79.9	1835.1	406.9	79.3	1989.8
	401.8	77.9	1918.6	412.2	86.6	1889.2
Mean recovery (%) ± IC (%)	95.5	93.4	94.8	95.7	95.1	94.9
%R.S.D. ($n=5$)	1.6	3.2	3.1	0.9	4.3	4.2

(v/v) provided the highest recovery rate for SMZ and TMP. The composition of the recovery solvent was decided based on the analyte solubilities at 25 °C. The solubility of SMZ is 0.5 mg/ml in water and 90.3 mg/ml in methanol [29]. The TMP solubility is 0.4 mg/ml in water and 12.1 mg/ml in methanol [30]. NP9 is soluble in water and ethanol [31]. Ethanol 96% (v/v) was used to obtain the best recovery results for NP9. The number of swabs used per sample was variable between two to three. Better recovery results were obtained when the spiked analytes were recovered by using three swabs per sample. Table 5 presents the recovery results for SMZ, TMP and NP9. Mean recovery results higher than 94% were obtained for all the analytes, fact demonstrating the suitability of the sampling method developed for cleaning validation. For every analyte, reproducible and comparative recovery values were obtained for both stainless steel and plexiglass surfaces.

The overall results obtained for method validation indicate that the method may be successfully used for the assay of SAM, SMZ, TBA, SAA and TMP in cleaning validation samples but it is less suitable for the analysis of NP9 at trace levels. In addition to the reasons related to the oligomeric nature of NP9 that detrimentally influences its quantification, its rather weak UV absorption constitutes an additional drawback. However the present method can be still used as a semi-quantitative indication of the level of detergent present on manufacturing equipment after cleaning.

4. Conclusions

Due to the composition of the co-trimoxazole cleaning validation sample neither HPLC, nor CZE methods could be developed for this study. Since NP9 is a non-ionic surfactant it was possible to obtain its separation, in the presence of the other sample compounds, through MEKC.

The advantages of the present method are derived from the complex separation mechanisms governing MEKC. Compounds that cannot be separated on the same RP-HPLC chromatographic column due to their diverse chemical nature can be separated through MEKC in the presence of a suitable background electrolyte. MEKC requires little setup time of the analytical equipment and could be suitable for the monitoring of equipment cleanliness in a routine-type working environment where fast turn-around of production is essential.

The MEKC method was developed, optimized and validated for the separation of the six potential contaminants likely to be found on the pharmaceutical manufacturing equipment, after the manufacture of co-trimoxazole. The electrophoretic separation was achieved within 14 min. The results obtained while validating the analytical method indicate that MEKC may be a useful technique for cleaning validation studies undertaken in the pharmaceutical manufacturing industry.

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References

[1] J.N. Delgado, W.A. Remers (Eds.), Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, 9th ed., J.B. Lippinkott, Philadelphia, PA, 1991, pp. 191–199.
[2] H.J. Kaiser, M. Minowitz, J. Valid. Technol. 7 (2001) 2–12.
[3] D. Liu, S. Hu, Q. Ding, Yaowu Fenxi Zazhi 11 (1991) 223–224.
[4] M.C. Ricci, R.F. Cross, J. Liq. Chromatogr. Relat. Technol. 19 (1996) 365–381.
[5] W.J. Zuo, K. Uchikura, Yaowu Fenxi Zazhi 17 (1997) 297–301.
[6] C. Akay, S.A. Ozkan, J. Pharm. Biomed. Anal. 30 (2002) 1207–1213.
[7] A.V. Pereira, Q.B. Cass, J. Chromatogr. B 826 (2005) 139–146.
[8] H. Amini, A. Ahmadiani, J. Pharm. Biomed. Anal. 43 (2007) 1146–1150.
[9] United States Pharmacopeia, 31st ed., United States Pharmacopeial Convention Inc., Rockville, MD, 2007, pp. 3298–3301.
[10] J.J. Bergh, J.C. Breytenbach, J.L. Dupreez, J. Chromatogr. 513 (1990) 392–396.
[11] B. Nickerson, S. Scypinski, H. Sokoloff, S. Sahota, J. Liq. Chromatogr. 18 (1995) 3847–3875.
[12] Guide to Inspections, Validation of Cleaning Processes, Food and Drug Administration, Office of Regulatory Affairs, Washington, DC, 1993, pp. 1–6.
[13] M.J. Shifflet, M. Shapiro, C. Levin, R. DeNisco, LC–GC 19 (2001) 312–317.
[14] K.D. Altria, T.A. Hadgett, Chromatographia 40 (1995) 23–27.
[15] Q.F. Li, H.Y. Zhang, H.W. Wang, X.G. Cheng, Z.D. Hu, Fenxi Huaxue 28 (2000) 793–797.
[16] J.W. Luo, H.W. Chen, S.W. Zhang, Fenxi Kexue Xuebao 16 (2000) 123–126.
[17] J.J.B. Nevado, G.C. Penalvo, F.J.G. Bernardo, J. Chromatogr. A 918 (2001) 205–210.
[18] K.A. Assi, K.D. Altria, B.J. Clark, J. Pharm. Biomed. Anal. 15 (1997) 1041–1049.
[19] L. Fan, L. Liu, H. Chen, X. Chen, Z. Hu, J. Chromatogr. A 1062 (2005) 133–137.
[20] A. Wang, F. Gong, H. Li, Y. Fang, Anal. Chim. Acta 386 (1999) 265–269.
[21] S. Lamba, S.K. Sanghi, A. Asthana, M. Shelke, Anal. Chim. Acta 552 (2005) 110–115.
[22] Q.X. Dang, Z.P. Sun, D.K. Ling, J Chromatogr. 603 (1992) 259–266.
[23] M.E.P. Hows, D. Perrett, J. Kay, J. Chromatogr. A 768 (1997) 97–104.
[24] A. Kunkel, S. Gunter, H. Watzig, Electrophoresis 18 (1997) 1882–1889.
[25] C.E. Lin, W.C. Lin, Y.C. Chen, S.W. Wang, J. Chromatogr. A 792 (1997) 37–47.
[26] G.L. Fourman, M.V. Mullen, Pharm. Technol. 17 (1993) 54–60.
[27] D.W. Layton, B.J. Mallon, D.H. Rosenblatt, M.J. Small, Reg. Toxicol. Pharmacol. 7 (1987) 96–112.
[28] L.J. Henderson, Am. J. Physiol. 21 (1908) 173–179.
[29] B.C. Rudy, B.Z. Senkowski, in: K. Florey (Ed.), Analytical Profiles of Drug Substances, vol. 2, Academic Press, New York, 1978, pp. 467–486.
[30] G.J. Manius, in: K. Florey (Ed.), Analytical Profiles of Drug Substances, vol. 7, Academic Press, New York, 1978, pp. 445–475.
[31] S. Budavari (Ed.), The Merck Index, 12th ed., Merck & Co., Whitehouse Station, NJ, 1996, p. 1146.