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Analysis of demeclocycline by capillary electrophoresis

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Abstract

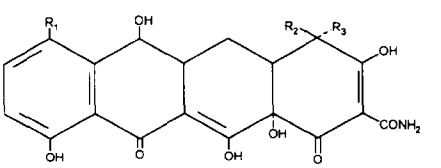
The analysis of demeclocycline and its main impurities by capillary electrophoresis is described. Demeclocycline is a tetracycline antibiotic. Main impurities are demethyltetracycline, 4-epidemeclocycline and 4-epidemethyltetracycline, which are formed due to fermentation or degradation. Method development was undertaken with a mixture consisting of demeclocycline and its main impurities already mentioned. Using an uncoated fused-silica capillary, the influence of buffer type, buffer pH and its concentration were systematically investigated. Non-ionic surfactant Triton X-100 was added to the running buffer which greatly improved separation selectivity. In all cases 1 m*M* EDTA was also added to prevent metal ion complexation. Then capillary temperature and applied voltage were also optimized. UV detection was performed at 254 nm. A relative standard deviation of 1.0% was obtained for demeclocycline. The limit of detection was 0.3% (signal-to-noise ratio=3) and the limit of quantification was 0.4% with respect to the original peak obtained with a solution containing 0.5 mg/ml demeclocycline.

Keywords: Demeclocycline; Antibiotics

1. Introduction

Demeclocycline (DMCTC) is a member of the tetracyclines, an important group of antibiotics, which are widely used. The structures of DMCTC and its impurities are shown in Fig. 1. DMCTC, like other tetracyclines undergoes epimerization at position C-4, resulting in the formation of 4-epidemeclocycline (EDMCTC). Demethyltetracycline (DMTC) is described as a fermentation impurity of DMCTC [1]. 4-Epidemethyltetracycline (EDMTC) is formed by the same process as that for EDMCTC.

Tetracycline drug analysis is often conducted using LC [2,3] as it offers excellent reproducibility and precision. However, the use of capillary electro-



	R_1	R_2	R_3
Demeclocycline (DMCTC)	Cl	H	$N(CH_3)_2$
Demethyltetracycline (DMTC)	H	H	$N(CH_3)_2$
4-Epidemeclocycline (EDMCTC)	Cl	$N(CH_3)_2$	H
4-Epidemethyltetracycline (EDMTC)	H	$N(CH_3)_2$	H

Fig. 1. Chemical structures of demeclocycline and its main impurities

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phoresis (CE) for separating tetracyclines has also gained considerable importance in recent years [4–9]. CE and its related methods, with features such as high efficiencies, high separation power and rapid analysis may be a viable alternative.

In this work, a capillary electrophoresis method is described. Non-ionic surfactant Triton X-100 is employed to improve separation selectivity due to the interaction with the negatively charged analytes. This detergent moves at the speed of electroosmotic flow and has to be distinguished from the pseudo-stationary phase in electrokinetic chromatography which has its own electrophoretic mobility. Triton X-100 would also not allow the separation of neutral compounds.

The method presented here enables the complete separation of DMCTC from its main impurities DMTC, EDMCTC and EDMTC and takes a slightly shorter analysis time (including the washing procedure) than LC. The results are compared with those of LC.

2. Experimental

2.1. Instrumental and operating conditions

Capillary electrophoresis was performed on Spectraphoresis 500 equipment (Thermo Separation Products, Fremont, CA, USA), coupled to a 3396 series II integrator (Hewlett-Packard, Avondale, PA, USA). Tetracyclines were detected by UV absorption at 254 nm. Injection was done hydrodynamically for 2 s. pH measurements were performed on a Consort pH-meter (Turnhout, Belgium) using calibration buffers constituted according to the European Pharmacopoeia [10]. When necessary, the pH of the running buffer was adjusted using 0.1 *M* NaOH before making up to volume. Electrophoretic parameters were determined using mixtures containing DMCTC, DMTC, EDMCTC and EDMTC. Throughout the study, all samples were dissolved in running buffer to obtain better peak symmetry.

2.2. Materials

All reagents were of analytical grade (Merck, Darmstadt, Germany or Acros Chimica, Geel, Belgium). House standards of DMCTC·HCl (98.8%), EDMCTC·HCl (96.5%) and DMTC dihydrate (97.2%), this content (m/m) being expressed in terms of the hydrochloride salt, were available. Small amounts of EDMTC·HCl were also prepared but its purity was not precisely determined. Commercial samples of DMCTC·HCl were obtained from one manufacturer. The fused-silica capillary was from Polymicro Technologies (Phoenix, AZ, USA). Throughout the study, Milli-Q^{50} water was used (Millipore, Milford, MA, USA). All the solutions were filtered through 0.2-μm nylon filters (Alltech, Laarne, Belgium).

3. Results and discussion

All development experiments were performed on an uncoated fused-silica capillary of 50 μm internal diameter (I.D.) and 38 cm effective length (l). Since selectivity greatly depends on the type of buffer [11], four different buffers were first compared, namely tris(hydroxymethyl)-aminomethane (Tris), sodium tetraborate, sodium carbonate and sodium phosphate. Each was prepared at a concentration of 20 m*M* and also contained 1 m*M* of sodium ethylenediaminetetraacetate (EDTA) to prevent interaction of the tetracycline structures with metals through complexation. The pH of all solutions was 11.50. Using Tris, the migration time was very short and it could not separate several substances of the mixture at all. Sodium tetraborate caused broadening of the DMTC and EDMTC peaks and migration time was more than 30 min. The last two buffers could separate the components of each of the two pairs of substances, DMCTC/EDMCTC and DMTC/EDMTC very well. However, under these conditions DMCTC and DMTC, and EDMCTC and EDMTC were not sufficiently separated. Subsequent experiments were performed with sodium phosphate because it gave the best separations. Parameters such as buffer pH, buffer concentration, temperature and applied voltage were optimised. The separation of the two pairs of substances, DMCTC/DMTC and EDMCTC/EDMTC was still very poor. When non-ionic surfactant Triton X-100 was introduced in the sodium phosphate buffer system, a clear improvement of the separation was observed. The influence

of several electrophoretic parameters was investigated further and results were as follows.

3.1. Influence of buffer pH

The pH is one of the most important parameters for improving selectivity in CE and small differences can cause the separation of closely related substances [11]. Keeping other conditions constant, the buffer pH was varied from 11.0 to 12.5 in steps of 0.25 pH unit. Since isoelectric points of tetracyclines are below the buffer pH, they are negatively charged and their electrophoretic mobility is opposite to electroosmosis. Results are shown in Fig. 2. The resolution of each pair of substances increased with a pH increase, which shows that an increase in electrophoretic movement of the substances has occurred, as negative ions have overcome the increase in electroosmosis. However, at about pH 12.25, the increase in electroosmosis became dominant and the resolution of DMCTC and DMTC started to decrease. Thus, pH 12.25 was chosen for further method development.

3.2. Influence of buffer concentration

The concentration of the buffer has a main influence on electroosmotic flow and current produced in the capillary [11]. It was varied from 20 to 70 m*M* in steps of 10 m*M*, keeping the EDTA concentration at 1 m*M*. With a concentration increase, the resolution of each pair increased continuously but the pair DMTC/EDMCTC was affected more than the two other pairs. Under normal conditions, as the buffer concentration is increased, the electrophoretic and electroosmotic mobilities are both decreased. The results show the former is reduced less with buffer concentration increase in this system. A concentration of 50 m*M* was finally chosen because it gave a current below 60 μA and good resolution for each pair of substances.

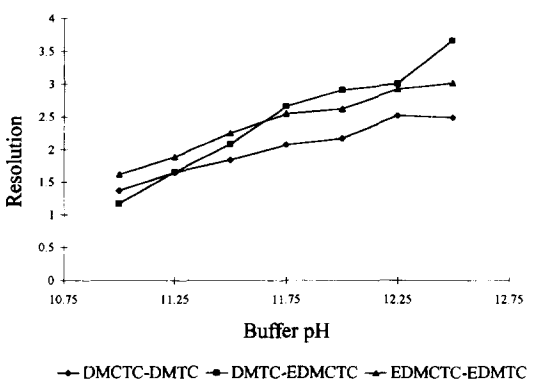

Fig. 2. Influence of buffer pH on the separation of demeclocycline and its main impurities. Capillary: uncoated fused silica, L=44 cm, l=38 cm, I.D.=50 μm; background electrolyte=sodium phosphate 50 m*M* including 0.35% (v/v) Triton X-100 and 1 m*M* EDTA; temperature=15°C; voltage=12 kV.

3.3. Influence of Triton X-100 concentration

Fig. 3 shows a graph of the migration time versus Triton X-100 concentration for all the substances. Triton X-100 concentration was varied from 0 to 0.40% (v/v) in steps of 0.05% (v/v). The migration order was DMCTC, DMTC, EDMCTC and EDMTC. The migration time of every substance decreased with Triton X-100 concentration increase in the buffer. The negatively charged tetracyclines have a higher overall mobility due to interaction with the nonionic micelles, which move with the speed of the electroosmotic flow. However, the migration time of DMCTC and EDMCTC decreased more than that of DMTC and EDMTC. This could be due to their higher hydrophobicity, if the analytes interact with the alkyl chain of Triton X-100 through hydrophobic interactions. The overall mobility of DMCTC and EDMCTC thus increases more with Triton X-100 concentration increase. Considering that DMCTC is the main component (>90%) in commercial samples, a Triton X-100 concentration of 0.35% was chosen because it gave suitable resolution for each pair of substances.

3.4. Influence of capillary temperature and applied voltage

Fig. 4 and Fig. 5 show the influence of capillary temperature and applied voltage, respectively. Selectivity changes with capillary temperature were small. Raising the temperature, separation of two pairs of components (including the main component) not only became worse but also current increased resulting in a worse repeatability. Voltage did not affect the selectivity much. As expected, a higher voltage

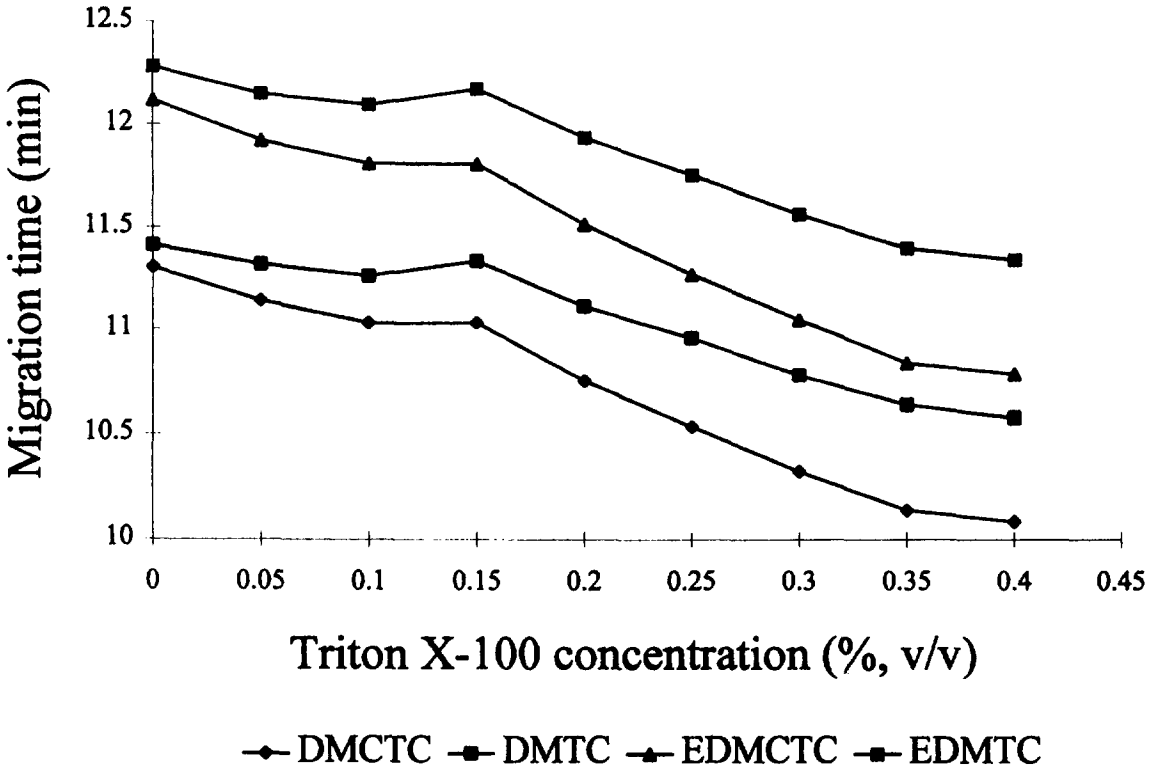

Fig. 3. Influence of Triton X-100 concentration on the separation of demeclocycline and its main impurities. Capillary: uncoated fused silica, L=44 cm, l=38 cm, I.D.=50 μm; background electrolyte=sodium phosphate (50 mM) including x % (v/v) Triton X-100 and 1 mM EDTA; pH=12.25; temperature=15°C; voltage=12 kV.

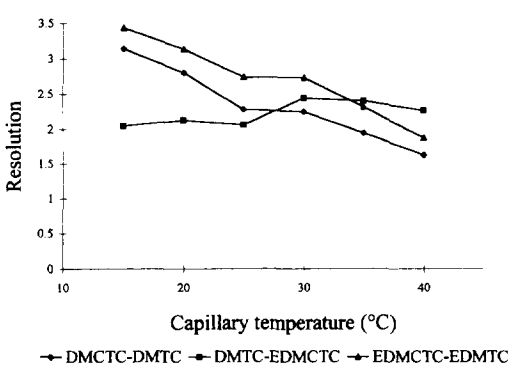

Fig. 4. Influence of capillary temperature on the separation of demeclocycline and its main impurities. Capillary: uncoated fused silica, L=44 cm, l=38 cm, I.D.=50 μm; background electrolyte=sodium phosphate (50 mM) including 0.35% (v/v) Triton X-100 and 1 mM EDTA; pH=12.25; voltage=12 kV.

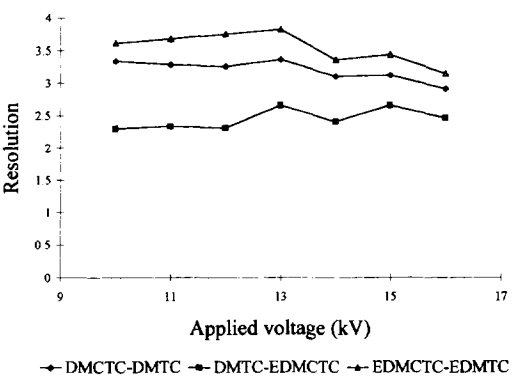

Fig. 5. Influence of applied voltage on the separation of demeclocycline and its main impurities. Capillary: uncoated fused silica, L=44 cm, l=38 cm, I.D.=50 μm; background electrolyte=sodium phosphate (50 mM) including 0.35% (v/v) Triton X-100 and 1 mM EDTA; pH=12.25; temperature=15°C.

shortened the run time, but increased current could result in an unstable method. From these results 15°C and 12 kV were chosen as optimal conditions.

It is clear from Figs. 2–4 that a combination of structural differences consisting of the presence of a chlorine atom together with epimerization, imparts a different behavior. Indeed, the pair DMTC/EDMCTC behave in a different way from the other two pairs. Fig. 6 shows a typical electropherogram. Running buffer was chosen as sample solvent, because it produced a better peak symmetry.

3.5. Quantitative analysis

The repeatability was checked with the same conditions as shown in Fig. 6. The sample used contained approximately 3.0% (m/m) of DMTC, 4.7% (m/m) of EDMCTC and 0.2% (m/m) of EDMTC. Relative standard deviations (R.S.D.) (n=9) were: 1.0% for DMCTC, 3.8% for DMTC, 2.0% for EDMCTC and 15% for EDMTC. This was almost as good as for LC where an R.S.D. value of 0.5% (n=38) was obtained for DMCTC [2]. The limit of detection (S/N=3) was 0.3% and the limit of quantification was 0.4% for DMCTC (n=7, R.S.D.=17%) with respect to the original peak obtained with a solution containing 0.5 mg/ml DMCTC. The following calibration line was obtained for DMCTC: $y=1864+190172x$, where y=corrected peak area and x=concentration of the analysed solution in mg/ml, r=0.9993, $S_{y,x}$ (standard error of estimate)=4371, investigated range=0.25-2.0 mg/ml, eight points (n=2).

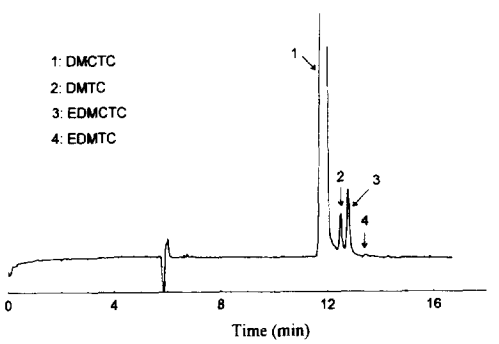

Fig. 6. Electropherogram of DMCTC and its main impurities. Capillary: uncoated fused silica, L=44 cm, l=38 cm, I.D.=50 μm; background electrolyte=sodium phosphate (50 mM) including 0.35% (v/v) Triton X-100 and 1 mM EDTA; pH=12.25; temperature=15°C, voltage=12 kV.

4. Conclusion

In this paper, the analysis of DMCTC by CE was investigated. From the results, it was found that satisfactory separation of DMCTC and its main impurities was achieved by CE. There should be great potential in the use of CE as an alternative tool for the analysis of demeclocycline.

Acknowledgments

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