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Capillary zone electrophoresis of minocycline[1]

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Abstract

A method is described for analysis of the tetracyline antibiotic minocycline using capillary zone electrophoresis. Potential impurities are 4-epiminocycline, 6-deoxy-6-demethyltetracycline, 7-didemethylminocycline, 7-monodemethylminocycline and 9-minocycline. Method development was undertaken with a mixture consisting of minocycline and its related substances mentioned above. Using a fused silica capillary, the type of buffer and its pH and concentration were investigated. In all cases 1 mM EDTA was added to prevent metal ion complexation. Instrumental parameters such as capillary temperature and applied voltage were optimised. The effects of the sample solvent and of organic modifiers in the buffer were also investigated. The following method is proposed: capillary: fused silica, $l = 38$ cm, $L = 44$ cm, 50 μm i.d.; buffer: 25 mM sodium tetraborate, 1 mM EDTA at pH 11.75; voltage, 13 kV; temperature, 15°C; UV detection performed at 254 nm. Relative standard deviations, linearity, LOD and LOQ are reported and compared with those of liquid chromatography.

Keywords: Assay; Capillary zone electrophoresis; Minocycline; Purity control

1. Introduction

Minocycline (MC) is a tetracycline antibiotic obtained by semi-synthesis from demeclocycline (DMCTC) [1]. 6-Deoxy-6-demethyltetracycline (6-DODMTC), 7-didemethylminocycline (7-DDMMC) and 7-monodemethylminocycline (7-MDMMC) are intermediates. 9-Minocycline (9-MC) is a synthetic side-product. In acid solution and upon storage MC is prone to epimerization, resulting in the formation of 4-epiminocycline (EMC). The structures of MC and its related substances are shown in Table 1.

Purity control of MC is mainly performed by liquid chromatography (LC) reversed-phase silica gel [2,3]. A LC method using poly(styrene-divinylbenzene) as the stationary phase has been published [4,5]. In this paper, a capillary zone electrophoresis (CZE) method is described. It enables the complete separation of MC from its related substances 7-DDMMC, 7-MDMMC, EMC, 6-DODMTC and 9-MC and the analysis time is shorter than with LC. Until now, no capillary electrophoresis (CE) method has been reported for resolving MC from its synthetic and degradation impurities.

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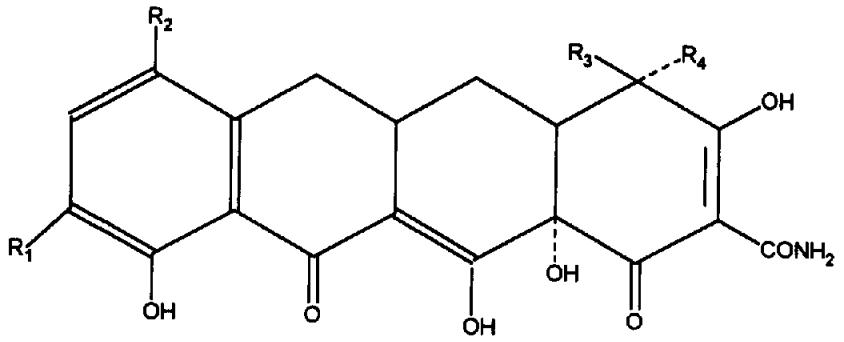
Table 1
Chemical structure of MC and its related substances



Compound	R_1	R_2	R_3	R_4
Minocycline (MC)	H	$N(CH_3)_2$	H	$N(CH_3)_2$
6-Deoxy-6-demethyltetracycline (6-DODMTC)	H	H	H	$N(CH_3)_2$
7-Didemethylminocycline (7-DDMMC)	H	NH_2	H	$N(CH_3)_2$
7-Monodemethylminocycline (7-MDMMC)	H	$NHCH_3$	H	$N(CH_3)_2$
9-Minocycline (9-MC)	$N(CH_3)_2$	H	H	$N(CH_3)_2$
4-Epiminocycline (EMC)	H	$N(CH_3)_2$	$N(CH_3)_2$	H

2. Experimental

2.1. Materials

All reagents were of analytical grade (Merck, Darmstadt, Germany or Acros Chimica, Geel, Belgium). A house standard of MC·HCl (91.0% w/w) was prepared in the laboratory. Reference substances of EMC·HCl (content not specified), 6-DODMTC·HCl (99.3% w/w), 7-DDMMC·HCl (content not specified) and 9-MC (92.1% w/w) were kindly donated by Lederle-Cyanamid (Louvain-la-Neuve, Belgium). Small quantities of 7-MDMMC were obtained by methylation of 7-DDMMC·HCl and isolated by a thin-layer chromatographic method previously described for identification of tetracyclines [6]. Fused silica capillary was from Polymicro Technologies (Phoenix, AZ). Throughout the study, Milli-Q^{50} water was used (Millipore, Milford, MA). All the solutions were filtered through 0.2 μm nylon filters (Alltech, Laarne, Belgium).

2.2. CE equipment and operating conditions

CE was performed on Spectraphoresis 500 equipment (Thermo Separation Products, Fremont, CA), coupled to a 3396 series II integrator (Hewlett-Packard, Avondale, PA). 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 [7]. When necessary, the pH of buffers was adjusted using 0.1 M NaOH before making up to volume. Electrophoretic parameters were determined using mixtures containing MC, EMC, 6-DODMTC, 7-DDMMC, 7-MDMMC and 9-MC. Throughout the study, all samples were dissolved in running buffer to obtain better peak symmetry.

3. Results and discussion

On starting the development of a method for the separation of MC and its main impurities, CZE was preferred because it is the simplest mode of CE. All development experiments were performed on uncoated fused silica capillary of 50 μm diameter and 38 cm effective length. Since selectivity depends to a great extent on the type of buffer [8], four different buffers were first compared, namely sodium tetraborate, dibasic sodium phosphate, sodium carbonate and tris(hydroxymethyl)aminomethane (Tris). Each was prepared at a concentration of 20 mM and also contained 1 mM of sodium edetate (EDTA) to prevent interaction of the tetracycline structures with metals through complexation. The pH of all solutions was 11.25. The Tris buffer did not separate several components of the mixture and the remaining three buffers only partly separated MC and its five related substances. Subsequent experiments were performed with sodium tetraborate because it performed best of all three buffers. The next parameter investigated was the pH. The pH is an important parameter for improving selectivity in CE and small differences can cause the separation of closely related substances. The alkaline region was selected to avoid sample adsorption on the capillary and epimerization of MC. The pH was varied between 10.5 and 12 in steps of 0.25 pH units. It was shown that only at pH 11.75 and 12 could separation of all six compounds be obtained. Fig. 1 shows a graph of the resolution vs. pH for all the pairs of substances. A pH of 11.75 was retained. Fig. 2 shows the influence of the buffer concentration. The concentration of the buffer influences the electroosmotic flow and the current produced in the capillary. It was varied from 20 to 40 mM in steps of 5 mM, keeping the EDTA concentration at 1 mM. A concentration of 25 mM was retained. Fig. 3 shows the influence of capillary temperature. Selectivity changes due to temperature were small and irregular. 15°C was chosen because on raising the temperature, the current increases so that repeatability of separation can be worse because of too much Joule heating. Fig. 4 shows the influence of applied

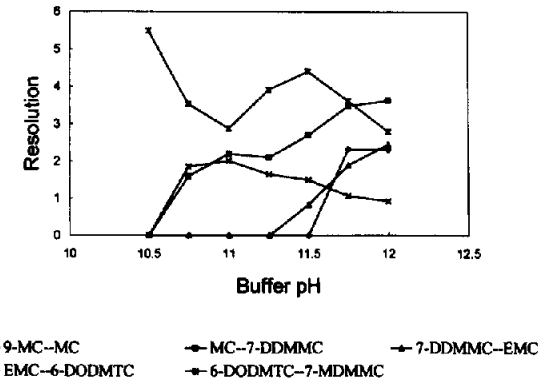


Fig. 1. Influence of buffer pH on separation of MC and its related substances. Capillary: uncoated fused silica, $L = 44$ cm, $l = 38$ cm, i.d. = 50 μm; background electrolyte = sodium tetraborate (25 mM)–EDTA (1 mM); voltage = 13 kV, temperature = 15°C.

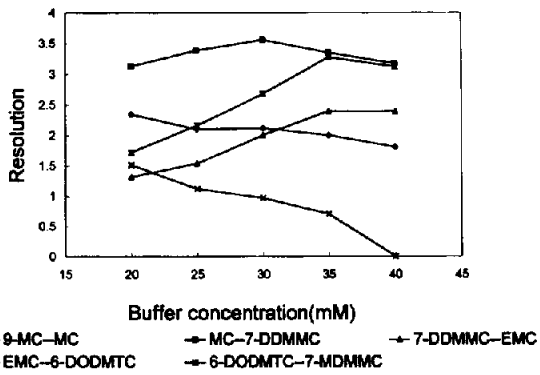


Fig. 2. Influence of sodium tetraborate concentration on separation of MC and its related substances. Capillary: uncoated fused silica, $L = 44$ cm, $l = 38$ cm, i.d. = 50 μm; background electrolyte = sodium tetraborate (x mM)–EDTA (1 mM); pH 11.75, voltage = 13 kV, temperature = 15°C.

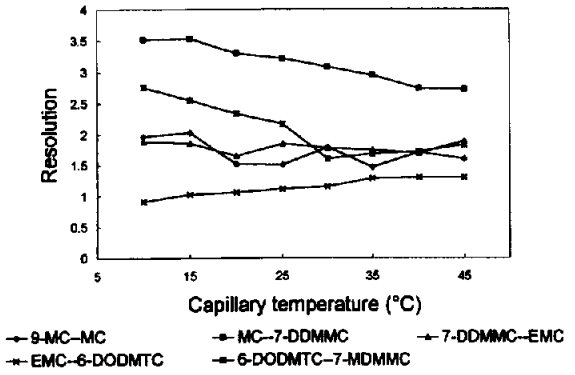


Fig. 3. Influence of capillary temperature on separation of MC and its related substances. Capillary: uncoated fused silica, $L = 44$ cm, $l = 38$ cm, i.d. = 50 μm; pH 11.75; background electrolyte = sodium tetraborate (25 mM)–EDTA (1 mM); voltage = 13 kV.

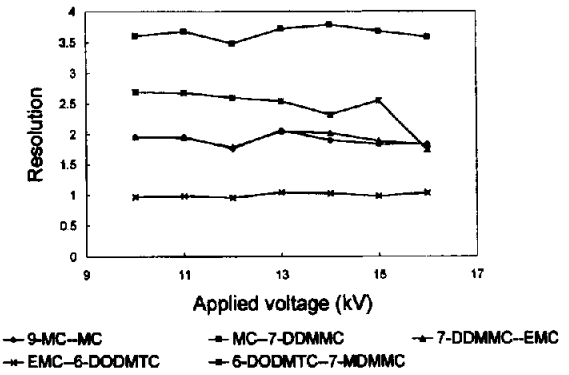


Fig. 4. Influence of applied voltage on separation of MC and its related substances. Capillary: uncoated fused silica, $L = 44$ cm, $l = 38$ cm, i.d. = 50 μm; pH 11.75; background electrolyte = sodium tetraborate (25 mM)–EDTA (1 mM); temperature = 15°C.

voltage. As expected, voltage did not change the selectivity much. A higher voltage shortens the run time, but increasing current results in an unstable method. To obtain better repeatability, 13 kV was selected. The effect of organic modifiers such as acetonitrile, methanol and 2-methyl-2-propanol was investigated. None of them provided improved resolution. Besides running buffer other sample solvents such as 0.01 M HCl, 0.01 M NaOH containing 0.1% w/v sodium sulfite, and buffer containing 0.1% w/v sodium sulfite were also investigated. Considering sample stability and peak symmetry, running buffer was selected as the sample solvent. Fig. 5 shows a typical electropherogram obtained with the final method. Comparison with the performance of LC [4] shows that the most apparent difference is the speed of CE with respect to LC. The latter analysis took about 30 min compared to 20 min for CE including the washing procedure.

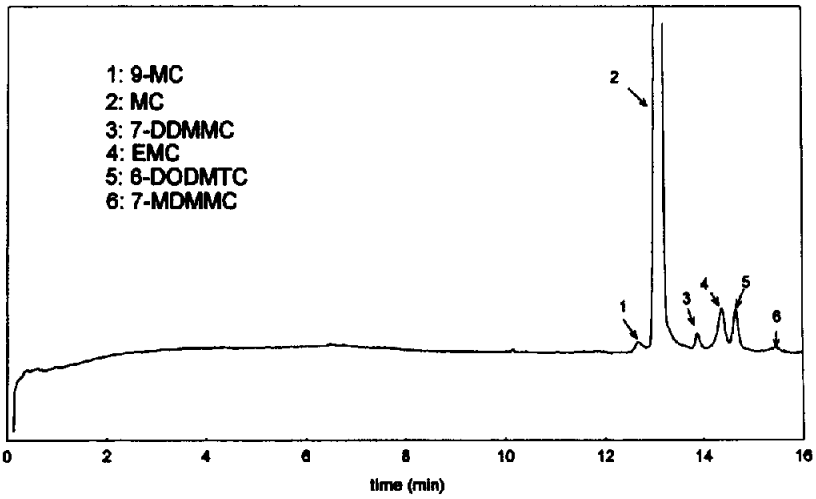


Fig. 5. Electropherogram of MC and related substances. Capillary: uncoated fused silica, $L = 44$ cm, $l = 38$ cm, i.d. = 50 μm; pH 11.75; background electrolyte = sodium tetraborate (25 mM)–EDTA (1 mM); temperature = 15°C, voltage = 13 kV.

Table 2
Quantitative data for CZE of minocycline (Capillary: uncoated fused silica, $L = 44$ cm, $l = 38$ cm, i.d. = 50μm; pH 11.75; background electrolyte, sodium tetraborate (25 mM)–EDTA (1 mM); temperature, 15°C; voltage, 13 kV)

Parameter	MC	EMC	6-DODMTC	9-MC	7-DDMMC	7-MDMMC
Content (%, w/w)	94.5	3.2	1.3	0.4	0.3	0.3
RSD (%) ($n = 7$, 0.5 mg ml^{-1})	2.4	4.2	6.1	13.3	16.7	22.8
LOD ($S/N = 2.5$)	10^{-4} mg ml^{-1} (0.02%)					
LOQ RSD = 15% ($n = 9$)	2×10^{-4} mg ml^{-1} (0.04%)					

Calibration curve (Y = corrected peak area, X = concentration of analysed solution in mg ml^{-1}): $Y = 4000 + 184144\ X$, $r = 0.9989$, $S_{y,x} = 5075$, range = 0.85–5.95 ng, seven points ($n = 2$)

The quantitative features of this method were examined and the results are shown in Table 2. As is generally the case, relative standard deviations (RSDs) are higher than with LC, and an RSD value of 0.8% ($n = 5$) was obtained for MC [4].

4. Conclusions

ACE method was developed for assay and purity control of MC. All the potential impurities are well separated from the main component and from each other. CE has advantages of simplicity and rapidity; LC performs better in quantitative analysis.

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