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Separation of Tetracycline and its Related Substances: comparison of Liquid Chromatography, Capillary Electrophoresis and Capillary Electrochromatography

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Tetracycline is a broad spectrum antibiotic extensively used in human medicine and as an animal feed supplement. Tetracycline (TC) can contain several impurities from fermentation (demethyltetracycline (DMTC), 2-acetyl-2-decarboxamidotetracycline (ADTC), chlortetracycline (CTC)) or from acid degradation (4-epitetracycline (ETC), 4-epianhydrotetracycline (EATC), anhydrotetracycline (ATC)). Due to the fact that some of these impurities are either inactive as an antibiotic or even toxic, their content is restricted in commercial bulk tetracycline. Because microbiological assays do not allow to determine these impurities, chromatographic methods are favored. A suitable analytical technique should be able to do a complete impurity profiling with minimal sample and reagent requirements in an acceptable time period.

Isocratic liquid chromatography on a poly(styrene-divinylbenzene) column of 25 cm × 0.46 cm ID is used to analyze official standards and commercial samples (European Pharmacopoelia (1997)). The mobile phase combines tert.-butanol (6.9 g/100 mL), 0.2 M phosphate buffer (10.0 mL), 0.02 M tetrabutylammonium sulphate (15.0 mL), 0.01 M sodium EDTA (10.0 mL) and water (up to 1000.0 mL) at a pH of 9.0 for elution at 60°C. The flow-rate is 1.0 mL/min and detection is achieved spectrophotometrically at 254 nm. This system allows the complete separation and resolution of TC, ETC, ATC, EATC and DMTC in a time under 40 min. Moreover, ADTC is also resolved from tetracycline. See Fig. 1 for a typical chromatogram.

The capillary zone electrophoretic system uses an uncoated fused silica capillary of 44 cm × 50 μm ID (Van Schepdael *et al.*, 1995). The background electrolyte is a sodium carbonate-EDTA (80 mM: 1 mM) mixture at pH 9.0 containing 0.5% MeOH. Injection is done hydrodynamically for 4 s, the detector is set at 270 nm, and the capillary is thermostatted at 15°C. This systems allows to separate the same impurities as the LC method, except for ADTC. Figure 1 shows a typical electropherogram. When the buffer concentration was raised to 120 mM and the pH was brought to 10.75, this fermentation impurity was also separated (Li *et al.*, 1997). Unfortunately, DMTC could not be separated under these circumstances, nor could CTC be quantified because it is unstable in the alkaline background electrolyte and converts partly to isochlortetracycline (ICTC).

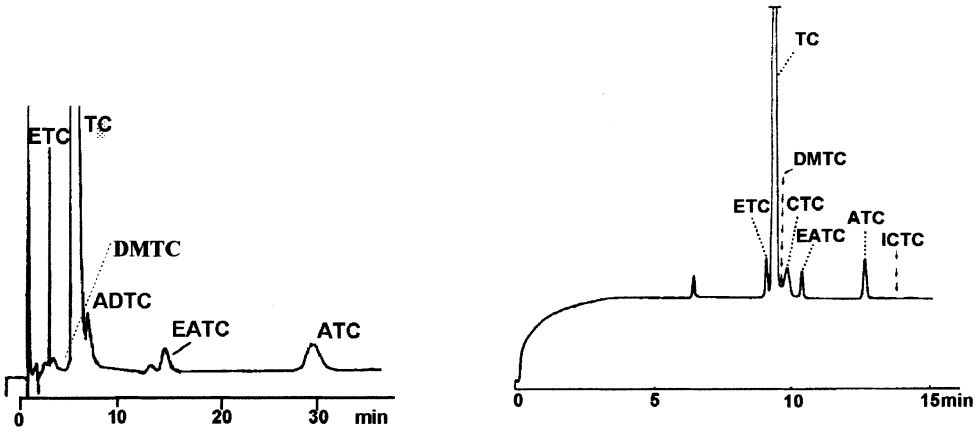

Figure 1. Comparison of the LC (left) and the CE (right) method as described in references 1 and 2, respectively. See text and Table 1 for experimental conditions.

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Table 1. Comparison of some of the properties of the LC and CE method

	LC (ref. 1)	CE (ref. 2)
Injected concentration	0.75 mg/mL	0.5 mg/mL
Injected volume	20 μL	15 nL
Injected mass	15 μg	7.5 ng
Linearity range tested for TC	0.6 – 1.0 mg/mL	0.2 – 0.7 mg/mL
y = peak area	$y = 919 + 9128\ x$	$y = 6204 + 401385\ x$
x = concentration in mg/mL		
r = correlation coefficient	$r = 0.9992$	$r = 0.9980$
$S_{y,x}$ = standard error of estimate	$S_{y,x} = 625$	$S_{y,x} = 3896$
Repeatability (RSD on peak area)	0.4% ($n = 38$)	1.0% ($n = 10$)
Speed (run time)	35 min	15 min
Detection wavelength	254 nm	270 nm
Reagent consumption: estimated number of assays with 100 mL buffer or mobile phase	3 (flow-rate = 1.0 mL/min)	50
LOQ (limit of quantification)	0.01%	0.4%
LOD (limit of detection)	Not tested	0.1%
Selectivity	Excellent	Very good, but ADTC is not separated from TC

Properties of the described LC and CE methods with respect to selectivity, speed, sample and reagent consumption, repeatability, linearity, detection and quantification limits are given in Table 1. The LC method is most appropriate if all impurities need exactly to be quantified. CE offers the advantage of a higher speed and a lower reagent consumption. With the aim of combining the best of the LC and CE techniques, it has been suggested to use capillary electrochromatography (CEC). These experiments are undertaken in capillary columns (CE format) packed with an LC type stationary phase (e.g. C_{18}). Applying an electric field across the length of the column generates an electroosmotic flow, making the mobile phase to move through the packed bed. After looking into recent literature on the subject (Yan *et al.*, 1995) and based on our initial experiments, it became clear that CEC is a powerful tool in the analysis of neutral compounds. However, the acid–base properties of tetracycline make it too polar in the pH range that is compatible with the stationary phase. On the one hand, a minimum of pH 3 is necessary to generate enough EOF and on the other hand, the octadecylsilyl packing material becomes unstable above pH 9. CEC of acidic compounds of pharmaceutical interest has only been described at pH 3.0 (Angus *et al.*, 1997), at which tetracycline is likely to degrade.

Further research in how to overcome this problem as well as how to eliminate bubble formation (pressurization) and how to make frits in a reproducible way are the subjects of future experiments. CEC might be the ideal technique if the quoted problems can be solved. Although it has not been tried out, it is possible that microbore LC will finally give us the satisfaction we are searching for.

REFERENCES

Angus, P. D. A., Stobaugh, J. F., Demarest, C. W., Payne, K. M., Sedo, K. R., Kwok, L. Y. and Catalano, T. (1997). Capillary Electrochromatography: Evaluation of Some Column Fabrication Techniques and Application to Pharmaceutical Analysis. *19th International Symposium on Capillary Chromatography and Electrophoresis,* 18–22 May 1997. Wintergreen, Virginia, USA.

Li, Y., Van Schepdael, A., Roets, E. and Hoogmartens, J. (1997). *J. Pharm. Biomed. Anal.*, **15,** 1063–1069.

Van Schepdael, A., Saevels, J., Lepoudre, X., Kibaya, R., Gang, N. Z., Roets, E. and Hoogmartens, J. (1995). *J. High Resol. Chromatogr.* **18,** 695–698.

Yan, C., Dadoo, R., Zhao, H., Zare, R. N. and Rakestraw, D. J. (1995). *Anal. Chem.* **67,** 2026–2029.

