

CE Analysis of Propranolol in Human Serum Using Dynamic Capillary Coating

Lilian Clohs and Angelina K. Winstanley,

Cardiome Pharma Corp., Vancouver, British Columbia, Canada.

A capillary electrophoresis method using the CELixir buffer system for the analysis of propranolol in human serum was compared with a method using phosphate buffer. While both methods showed good linearity over the concentration range tested, the CELixir method showed better accuracy and precision at the low concentration end (25–50 ng/mL). The limits of quantitation and detection of the CELixir method were 25 and 12.5 ng/mL, respectively. Improved migration-time reproducibility was obtained with the CELixir method, which was probably responsible for an enhanced overall performance compared with the standard phosphate method.

Introduction

Capillary electrophoresis (CE) is gaining wider acceptance in analytical laboratories and has become an important tool for routine analysis in the pharmaceutical and biotech industries¹. Specifically in the area of bioanalytical chemistry several publications report the use of CE for the analysis of drugs in biological matrices. The applications include drug quantifications for pharmacokinetic and metabolism studies,^{2,3} *in vitro* drug metabolism studies,^{4,5} as well as applications in forensic analysis⁶ and therapeutic drug monitoring.⁷ Methods can be validated and show good accuracy and precision.^{8,9}

CE at Cardiome Pharma Corp. has been adopted as the method of choice for the analysis of drugs in biological matrices, such as plasma, brain, urine and bile. The technique allows fast method development and method transfer with low operational costs. Indeed, a single set of electrophoretic conditions is used in our laboratories to analyse structurally diverse compounds of our antiarrhythmic amine series without the need for method optimization.

Our standard CE method uses a simple phosphate buffer (pH 2.5) and uncoated silica capillaries for the analysis of basic drugs after a liquid–liquid extraction of compounds from the biological matrix. The

method shows good accuracy and precision over a broad concentration range (typically 75–10 000 ng/mL for plasma samples), but an improvement in concentration sensitivity would be desirable for the determination of very low drug plasma concentrations, such as those encountered in pharmacokinetic studies. In our experience, CE analysis of basic drugs in plasma at concentrations less than 75 ng/mL often suffers from poor accuracy and precision. A possible cause could be inconsistencies in the electroosmotic flow (EOF) between runs resulting in non-reproducible migration times. Because the peak area in CE is proportional to the migration time, variability in the latter parameter could lead to low precision in peak area estimates. Another factor affecting precision could be adsorption of amine compounds to the silanol groups on the capillary wall producing tailing and, therefore, affecting peak shape and consequently integration.

Wall-coated capillaries can be used to mask the silanol groups on the capillary surface and therefore eliminate or modify the EOF and reduce the interaction of the solutes with the capillary wall. Permanent coating can be obtained either by covalent bonding of agents such as polyacrylamide, polyvinylpyrrolidinone or octadecylsilane to the capillary surface or by adsorption of

materials (e.g., poly[vinyl alcohol]) to the capillary wall. This has been shown to produce efficient and reproducible separations.^{10–13} Belder and colleagues reported effective reduction of protein adsorption and good migration-time reproducibility using a permanent coating with poly(vinyl alcohol) and glutaraldehyde as a cross-linking agent.¹⁴

Dynamic coating can be obtained by introducing additives to the background electrolyte or by rinsing the capillary before runs with a surface modifier. For example, cationic surfactants such as cetyltrimethylammonium bromide or hexadecyltrimethylammonium bromide when added to the buffer can reduce the EOF and limit solute adsorption.¹⁵ This is because the monomers adhere to the wall through ionic interactions masking the ionized silanol groups. A second layer of the surfactant may adsorb through hydrophobic interactions resulting in a net positive charge on the capillary surface and consequent reversal of the EOF. This mechanism was also proposed by Thornton et al.¹⁶ to explain the EOF reversal produced by alkanesulfonic acids, such as ethanesulfonic acid and octanesulfonic acid, added to the run buffer. Ethanesulfonic acid has also been reported to produce sharper peaks for quinidine and procainamide, possibly by reducing the

In our experience, CE analysis of basic drugs in plasma at concentrations less than 75 ng/mL often suffers from poor accuracy and precision.

wall adsorption of these two basic drugs.¹⁷ Dynamic coating with polybrene and poly(vinylsulfonate) introduced in the run buffer resulted in consistent EOF and good migration-time reproducibility for a series of basic compounds.¹⁸ Improved detection limits were also reported when testing the optical purity of drug enantiomers using a polyamine-coated capillary, possibly through decreased peak tailing because of reduced drug adsorption to the capillary surface.¹⁹

A dynamic coating system has been developed by Analis SA, Belgium, and is commercialized under the name CElixir or CEofix. This system consists of two buffers, which produce a dynamic coating of the capillary surface resulting in uniform EOF and decreased adherence to the capillary wall. A buffer containing polycations is injected first to form a positively charged layer on the capillary surface. A second solution consisting of polyanions is then introduced. The polyanions adsorb to the positively charged layer and form a highly negatively charged layer, which is insensitive to pH changes, resulting in a strong and constant EOF.

A few recent papers have reported improvements with the use of the CElixir buffer. Altria reported improved migration-time reproducibility and peak shape in the analysis of basic drugs using this buffer system.²⁰ Enhanced performance with CElixir was also reported in clinical chemistry applications^{21,22} and for the screening of basic compounds in toxicological²³ and forensic analysis.²⁴

In this article, we compare our standard analytical method using phosphate buffer with a method using the CElixir system for the analysis of propranolol in human serum. Propranolol was used as the test compound because it is a basic drug, so that the assay will be transferable to other basic molecules in Cardiome's library. The internal standard (IS) was RSD921, the original lead compound for the antiarrhythmic programme and structurally similar to the other compounds in this series (Figure 1).

Objective

The objective of this study was to compare the performance of a CE method using the CElixir buffer with our standard assay using phosphate buffer, for the analysis of a basic

drug (propranolol) in human serum. The linearity, accuracy and precision of the two methods were compared. Migration-time reproducibility, analysis time and efficiency were also assessed.

Experimental

Materials: Human serum and D,L-propranolol (hydrochloride salt) were obtained from Sigma (St. Louis, Missouri, USA). The IS, RSD921 (hydrochloride salt), was synthesized at Cardiome (Vancouver, British Columbia, Canada). Chem Elut cartridges (1 mL) were purchased from Varian (Harbor City, California, USA). CElixir initiator solution (A) and accelerator solution (B), and fused-silica capillaries for the CE analysis were obtained from MicroSolv Technology Corp. (Long Branch, New Jersey, USA). All other reagents were at least of analytical grade.

Standard curve preparation and extraction procedure: Standard solutions of propranolol in water (40 μ L) were added to blank human serum (0.2 mL) to obtain final concentrations of propranolol in serum ranging from 12.5 to 1000 ng/mL. Quality control samples at low (25 and 50 ng/mL) and high (500 ng/mL) concentrations were prepared to assess the accuracy and precision of the method. IS (50 μ L of a 4 μ g/mL solution of RSD921 in water) was added to all samples. Proteins were precipitated with the addition of 50 μ L 10% trichloroacetic acid and the pH was subsequently raised (pH~12) with 1 M NaOH. Samples were transferred to Chem Elut cartridges and extracted with ether (2 \times 3 mL). The ether was evaporated to dryness under a stream of nitrogen and the dry extracts were reconstituted in 40 μ L of 10 mM phosphate buffer (pH 2.5) for analysis by CE.

CE conditions: CE analyses were performed on a P/ACE System MDQ (Beckman Coulter, Fullerton, California, USA) with UV detection at 214 nm. Uncoated silica capillaries with 60 cm length (50 cm to detector) and 75 μ m internal diameter were used. For the standard CE method, the run buffer used was 100 mM sodium phosphate (pH 2.5) and the separation voltage was 25 kV (normal polarity). The capillary cartridge temperature was maintained at 20 °C. Samples were injected by pressure at 1 psi for 10 s, with a postinjection water plug of

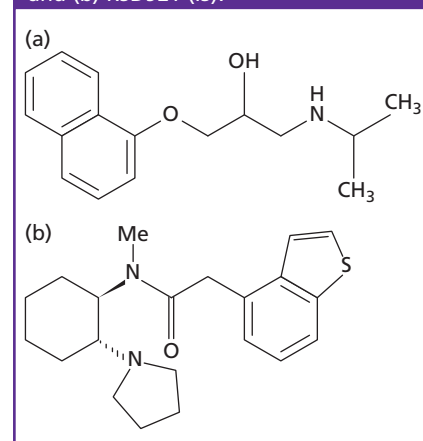
0.1 psi for 10 s. The capillary was washed after each run with a series of rinses at 20 psi: water (0.5 min), methanol (1 min), water (0.5 min), 0.1 M NaOH (1 min), water (0.5 min) and run buffer (1 min). For the CElixir method, the same capillary, cartridge temperature, detection wavelength and sample injection were used. The capillary was rinsed prior to each run for 1 min with 0.1 M NaOH, 1 min with CElixir initiator solution (A) and 2 min with CElixir accelerator solution (B) (pH 2.5) at 20 psi, and the separation voltage was 15 kV (normal polarity). Corrected peak areas (area/migration time) were used for the calculations.²⁵

Results

Good linearity ($r^2 \geq 0.992$, with 1/concentration weighting) was obtained for the CE analysis of propranolol in human serum within the 25–1000 ng/mL concentration range using both the standard phosphate and the CElixir buffers (Table 1). The back-calculated concentrations for the standards showed acceptable deviation from the nominal values, although the deviations were generally larger for the analyses performed using the standard phosphate buffer, compared with the CElixir buffer. The limit of detection (LOD) of the method was 12.5 ng/mL of propranolol in serum, with a signal-to-noise ratio of 3:1. No interference from the matrix was observed as the analyses of a blank serum extract using phosphate buffer or CElixir buffer produced electropherograms free of interfering peaks.

To assess the accuracy and precision of the CE assays, triplicate QC samples of propranolol in serum at three different concentrations were prepared and analysed using the 100 mM phosphate buffer

Figure 1: Structures of (a) propranolol and (b) RSD921 (IS).



Peak shape was improved with the CELixir method as revealed by the increase in theoretical plate number calculated for both propranolol and the IS...

(pH 2.5) and the CELixir buffer (pH 2.5). The results are presented in Table 2 and show good accuracy ($\leq 16\%$) and precision ($\leq 3\%$) for the two low (25 and 50 ng/mL) QCs after analysis using the CELixir buffer

system. The high (500 ng/mL) QC samples showed excellent accuracy ($\leq 9\%$) and precision (4%) using the same buffer. The limit of quantitation (LOQ) of the method for the analysis of propranolol in serum

was 25 ng/mL using the CELixir buffer.

Analysis of the QC samples using the standard phosphate method (Table 2) resulted in poor accuracy ($\leq 23\%$) and precision ($\leq 23\%$) at the low concentration end (25 and 50 ng/mL), while the accuracy ($\leq 17\%$) and precision ($\leq 7\%$) were acceptable at the high (500 ng/mL) QC level. Conventionally, accuracy expressed as $\pm 15\%$ ($\pm 20\%$ at LOQ) deviation from the nominal

Table 1: Corrected peak area ratio (CAR) propranolol/IS, line parameters, back-calculated concentrations and deviations for the CE analysis of propranolol in human serum using 100 mM phosphate (pH 2.5) and CELixir (pH 2.5). Weighting 1/x was used.

Propranolol concentration (ng/mL)	100 mM Phosphate Buffer (pH 2.5)			CELixir Buffer (pH 2.5)		
	CAR	Calculated concentration (ng/mL)	% Deviation from nominal	CAR	Calculated concentration (ng/mL)	% Deviation from nominal
25	0.0672	27	9	0.0474	27	7
50	0.1295	49	-3	0.1268	55	10
75	0.2556	92	23	0.2121	85	13
100	0.2763	99	-1	0.2436	96	-4
250	0.5058	178	-29	0.4325	163	-35
500	1.2902	449	-10	1.4392	518	4
1000	3.1917	1105	10	2.9642	1057	6
Slope	2.899			2.832		
Intercept	-0.0116			-0.0283		
r ²	0.992			0.993		

Table 2: QC performance for the CE analysis of propranolol in human serum using 100 mM phosphate (pH 2.5) and CELixir (pH 2.5).

	100 mM Phosphate buffer pH 2.5			CELixir buffer pH 2.5		
	Concentration (ng/mL)	Deviation (%)	CV (%)	Concentration (ng/mL)	Deviation (%)	CV (%)
QC low A			12			2
Nominal	25			25		
Calculated value 1	31	23		29	15	
Calculated value 2	25	-1		28	11	
Calculated value 3	25	1		28	13	
Average	27	8		28	13	
QC low B			23			3
Nominal	50			50		
Calculated value 1	62	23		55	10	
Calculated value 2	38	-23		54	8	
Calculated value 3	53	7		58	16	
Average	51	2		56	11	
QC high			7			4
Nominal	500			500		
Calculated value 1	505	1		544	9	
Calculated value 2	586	17		499	0	
Calculated value 3	562	12		515	3	
Average	551	10		519	4	

and precision (expressed as coefficient of variation, CV%) of $\leq 15\%$ ($\leq 20\%$ at LOQ) are accepted when validating bioanalytical methods.²⁶ The results suggest that the LOQ for the analysis of propranolol in serum using the standard phosphate method is > 50 ng/mL.

Figure 2 shows representative electropherograms of standards of propranolol in serum analysed using the standard phosphate and the CELixir methods. A more stable and less noisy baseline was observed for the analyses using the CELixir buffer compared with the method using phosphate. Considerable variability in migration times was observed for the runs using standard phosphate buffer while the CELixir analyses resulted in very reproducible migration times. The CELixir buffer also allowed shorter analysis times: the IS (last peak) migrated past the detector at about 13 min while it appeared at about 18 min when the phosphate buffer was used. Shorter migration times were obtained despite the fact that a much lower voltage was used with the CELixir method. This was probably caused by a faster EOF. A twofold decrease in the migration times was described by Boone and colleagues when analysing a series of drugs using a CELixir buffer compared with a regular phosphate buffer method.²³

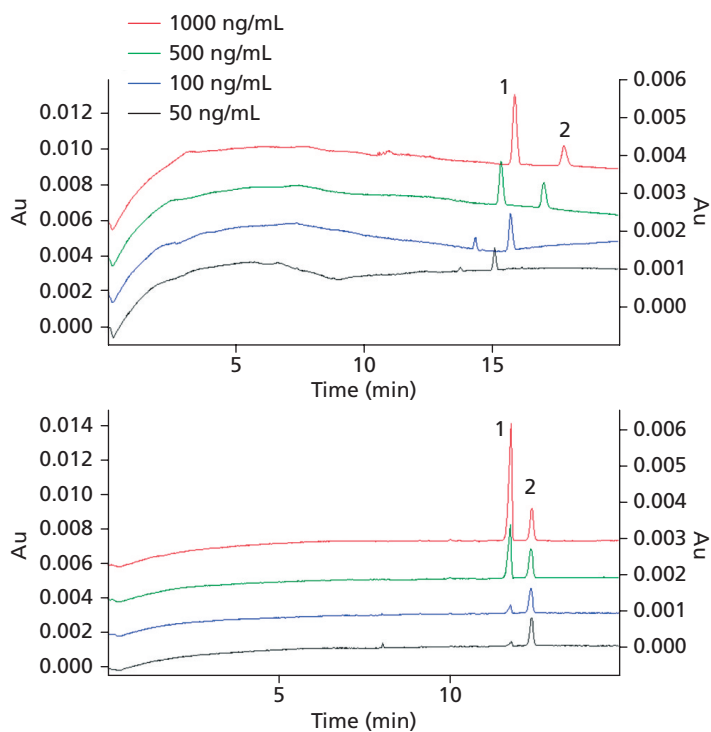
Table 3 shows the migration-time reproducibility for the analyses of the complete set of samples ($n = 17$, 8 standards and 9 QC samples) using the two different buffer systems. The migration time showed more variability for propranolol and the IS ($CV \leq 6\%$) using phosphate buffer although the relative migration time was considerably better ($CV = 0.7\%$). The CV% was consistently low for both the propranolol and the IS peak ($CV = 0.2\%$) as well as for the relative migration time ($CV = 0.2\%$) when the CELixir buffer was used. Good migration-time precision was also reported by Altria,²⁰ and Lurie and co-workers²⁴ for a series of injections of basic drugs analysed using the CELixir buffer system.

Peak shape was improved with the CELixir method as revealed by the increase in theoretical plate number calculated for both propranolol and the IS compared with the values obtained after analysis using phosphate buffer (Table 4).

Conclusions

The CE method for the analysis of propranolol in human serum using the CELixir buffer system showed good linearity, accuracy and precision within the

Figure 2: CE analysis of propranolol in human serum. Electropherograms of extracted calibration standards analysed using (a) 100 mM phosphate buffer (pH 2.5) and (b) CELixir buffer (pH 2.5) (CE conditions: see text).



Peaks: 1 = propranolol, 2 = internal standard.

Table 3: Migration-time reproducibility for the CE analysis of propranolol in human serum using 100 mM phosphate (pH 2.5) and CELixir (pH 2.5).

Migration Time Reproducibility	100 mM Phosphate pH 2.5 CV (%)	CELixir pH 2.5 CV (%)
Propranolol	5	0.2
IS	6	0.2
Migration-time ratio	0.7	0.2

Table 4: Comparison of efficiency obtained for the CE analysis using 100 mM phosphate (pH 2.5) and CELixir (pH 2.5).

Concentration (ng/mL)	Compound	Number of theoretical plates	
		100 mM Phosphate pH 2.5	CELixir pH 2.5
50	Propranolol	49490	110168
	IS	47039	67251
100	Propranolol	85186	123452
	IS	65475	61863
500	Propranolol	53705	122400
	IS	46187	56336
1000	Propranolol	42337	84103
	IS	33976	64508

25–1000 ng/mL concentration range. The LOQ of the CElixir method was 25 ng/mL and the LOD was 12.5 ng/mL. Migration times for propranolol and the internal standard were shorter and very reproducible. Because peak area is related to the migration time in CE, it is probable that the improved migration-time reproducibility, as well as lower adsorption to the capillary wall conferred by the dynamic coating resulted in enhanced overall performance of the CElixir method compared with the standard phosphate method.

Acknowledgement

We would like to thank Dr Kevin D. Altria for suggesting the use of the CElixir buffer.

References

1. K.D. Altria, A.B. Chen and L. Clohs, *LC•GC Eur.*, **14**(12), 736–744 (2001).
2. M.R. Hadley, P. Camilleri and A.J. Hutt, *Electrophoresis*, **21**, 1953–1976 (2000).
3. D. Levêque et al., *J.Chromatogr. B*, **697**, 67–75 (1997).
4. L. Clohs and J. Wong, J. Cap. *Electrophor.*, in press.
5. D.P. Bogan et al., *Xenobiotica*, **26**(4), 437–445 (1996).
6. G. Manetto, F. Crivellente and F. Tagliaro, *Ther. Drug Monit.*, **22**, 84–88 (2000).
7. Z.K. Shihabi, *J. Chromatogr. A*, **807**, 27–36 (1998).
8. L. Clohs and K.M. McErlane, *J. Pharm. Biomed. Anal.*, **24**, 545–554 (2001).
9. W. Reeves Huie et al., *J.Chromatogr. B*, **693**, 451–461 (1997).
10. R. Kuldvee and W. Thormann, *Electrophoresis*, **22**, 1345–1355 (2001).
11. K.A. Assi, B.J. Clark and K.D. Altria, *Electrophoresis*, **20**, 2723–2725 (1999).
12. M. Gilges, M.H. Kleemiss and G. Schomburg, *Anal. Chem.*, **66**, 2038–2046 (1994).
13. X.W. Yao, D. Wu and F.E. Regnier, *J. Chromatogr.*, **636**, 21–29 (1993).
14. D. Belder et al., *Electrophoresis*, **22**, 3813–3818 (2001).
15. M. Siluveru and J.T. Stewart, *J. Pharm. Biomed. Anal.*, **15**, 1751–1756 (1997).
16. M.J. Thornton, J.S. Fritz and C.W. Klampfl, *J. High Resol. Chromatogr.*, **20**, 647–652 (1997).
17. W. Ding and J.S. Fritz, *Anal. Chem.*, **70**, 1859–1865 (1998).
18. L. Bendahl, S.H. Hansen and B. Gammelgaard, *Electrophoresis*, **22**, 2565–2573 (2001).
19. K.A. Assi et al., *J. Chromatogr. A*, **817**, 83–90 (1998).
20. K.D. Altria, *J. Chromatogr. A*, in press.
21. N. Mario et al., *Clin. Chem.*, **45**, 285–288 (1999).
22. B. Wuyts et al., *Clin. Chem.*, **47**, 247–255 (2001).
23. C.M. Boone et al., *J. Chromatogr. A*, **927**, 203–210 (2001).
24. I.S. Lurie et al., *J. Forensic Sci.*, **46**(5), 1025–1032 (2001).
25. K.D. Altria, *Chromatographia*, **35**(3/4), 177–182 (1993).
26. V.P. Shah et al., *Eur. J. Drug Metab. Pharmacokinet.*, **16**(4), 249–255 (1991).

Cardiome Pharma Corp., 3650 Wesbrook Mall, Vancouver, British Columbia, V6S 2L2, Canada, e-mail: clohs@cardiome.com. Her main area of research focuses on the development and implementation of CE methods for the analysis of drugs in biological matrices, in support of drug discovery activities.

Angelina K. Winstanley is a Research Associate in the Bio-Analytical Chemistry Department.

“CE Currents” editor **Kevin D. Altria** is senior principal scientist in the pharmaceutical development group at GlaxoSmithKline R&D, Ware, Hertfordshire, UK, and is a member of the Editorial Advisory Board of *LC•GC Europe*. Direct correspondence about this column to “CE Currents,” K.D. Altria, Pharmaceutical Development, GlaxoSmithKline, Park Road, Ware, Hertfordshire SG12 0DP, UK, fax +44 1920 882 295, e-mail: kda8029@GlaxoWellcome.co.uk, website: <http://www.ceandcec.com/>

Lilian Clohs is Associate Director of the Bio-Analytical Chemistry Department at

