ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
20 (1999) 179–183

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Separation of morphine and its oxidation products by capillary zone electrophoresis

Bohumil Proksa *

Slovakofarma a.s., SK-920 27 Hlohovec, Slovak Republic

Received 4 May 1998; received in revised form 16 July 1998; accepted 14 August 1998

Abstract

Separation of morphine and its oxidation products (10-*S*-hydroxymorphine, pseudomorphine and morphine *N*-oxide) by capillary zone electrophoresis in Tris–borate buffer in the presence of cyclodextrins was studied. Pyridoxine was used as an internal standard.

Keywords: Morphine; Pseudomorphine; 10-*S*-hydroxymorphine; Morphine *N*-oxide; Capillary zone electrophoresis; Cyclodextrins

1. Introduction

Morphine (**1**) is oxidized into pseudomorphine (**2**) and morphine *N*-oxide (**3**) in aqueous solutions [1]. This degradation, for which a free radical mechanism was proposed, is dependent on pH and concentration of dissolved oxygen [2]. Oxidation of morphine in solid phase proceeds probably according to another mechanism, because 10-*S*-hydroxymorphine (**4**) and traces of 10-oxomorphine were identified in stored crystalline morphine sulfate [3] and morphine base [4], respectively.

Capillary electrophoresis was used for separation and determination of morphinane alkaloids in various matrices—in pharmaceutical formulations [5], in opium [6], bound as glucuronides in body fluids [7], or in samples of forensic interest [8–10]; however, we did not find any mention of separation of morphine, its *N*-oxide, pseudomorphine and 10-*S*-hydroxymorphine, which we identified as the main impurities in crystalline morphine or its salts. This paper deals with separation of the aforementioned compounds by capillary zone electrophoresis in the presence of cyclodextrins.

2. Experimental

2.1. Drugs and chemicals

Morphine and codeine (**5**) were from Slovakofarma a.s. Hlohovec; pseudomorphine (**2**) [11] and morphine *N*-oxide (**3**) [12], were synthesized, 10-*S*-hydroxymorphine (**4**) and 2,2′-biscodeine (**6**) (Fig. 1) were isolated in our laboratory. Prepared

* Fax: +421-804-736-2077.
E-mail address: proksab@slofa.sk (B. Proksa)

0731-7085/99/$ - see front matter
PII: S0731-7085(99)00015-1

and isolated compounds were fully characterized by spectral methods. β-Cyclodextrin (β-CD), (2-hydroxy-propyl)-β-cyclodextrin (HP-β-CD), (2-hydroxypropyl)-γ-cyclodextrin (HP-γ-CD), γ-cyclodextrin (γ-CD), were from Fluka AG, (Buchs, Switzerland); heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) was purchased from Sigma, St. Louis, USA. All other chemicals were obtained from Fluka.

2.2. Apparatus

A HP 3D Capillary Electrophoresis apparatus (Hewlett Packard, Waldbronn, Germany) with a diode array detector (190–600 nm) operated at 210 nm was used for analysis; data were processed on a HP ChemStation. Capillaries: an untreated fused silica capillary tubes 48.5 and 64.5 cm (effective length 40 and 56 cm, respectively) with 50 mm ID and extended light path (× 3) as well as the 64.5 cm × 50 mm capillary tube coated with polyvinylalcohol (PVA) (Hewlett Packard, Waldbronn, Germany) were used. Prior to use, the bare silica capillary was rinsed with 1 M NaOH (15 min), distilled water (10 min) and the BGE (100 mM Tris–phosphate, pH 2.8, 5 min); PVA coated capillary was rinsed with 20 mM phosphoric acid (20 min), distilled water (10 min) and the buffer (5 min). Between analyses both capillaries were flushed with 10 mM phosphoric acid (2 min), distilled water (1 min) and buffer solution (3 min). Samples were kept at laboratory temperature in the autosampler and pressure injected at 5 kPa for 2 s. Resolution of peaks (R_s) was calculated according to the equation

$$R_s = 2(t_2 - t_1)/(w_1 + w_2)$$

where t is the migration time, w the baseline peak width. Volume of sample loaded was calculated from the Hagen–Poiseuille equation

$$V = \Delta P \cdot d^4 \cdot \pi \cdot t \cdot (128 \cdot \eta \cdot \mathrm{L})^{-1}$$

where V is the volume, ΔP the pressure difference across the capillary, d the capillary inside diameter, t the time, η the buffer viscosity, L the total capillary length.

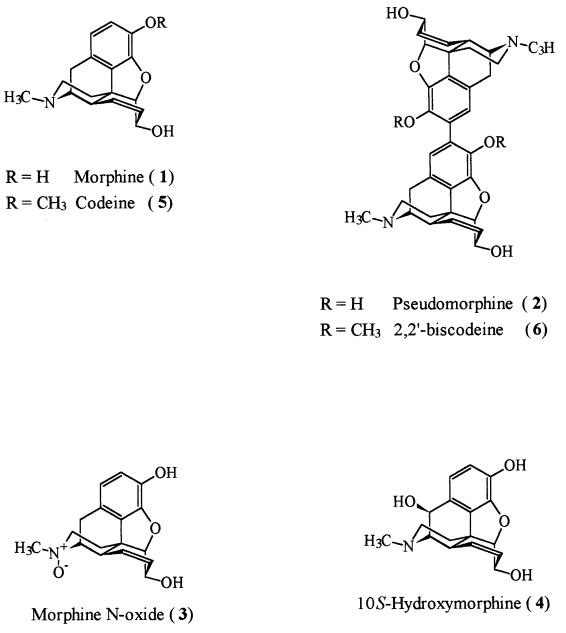

Fig. 1. Structure of studied morphinane alkaloids.

2.3. Sample preparation

The background electrolyte consisted of Tris–phosphate (pH 2.6, 100 mM) buffer completed with cyclodextrin. The sample solutions was prepared by dissolving 2–5 mg of the selected compound in 2 ml of 0.1 M aqueous HCl and filling up to 10 ml with distilled water and before pressure injection (100 kPa · s) it was filtered through a 0.20 mm nylon membrane filter.

3. Results and discussion

Peaks of morphine (**1**) and pseudomorphine (**2**) were excellently separated in the bare silica capillary or in the PVA coated one in plain BGE and the addition of β-cyclodextrin or its derivatives further enhanced the resolution (Table 1). However, there was very low or no difference in interaction of morphine and pseudomorphine with γ- or HP-γ-CD, respectively, and peaks of these compounds were not resolved. The same phenomenon was observed also with pair codeine (**5**) and 2,2′-biscodeine (**6**). Separation of morphine and 10-*S*-hydroxymorphine (**4**) was very high in

Table 1
Resolution (R_s) of peaks of morphine (**1**), pseudomorphine (**2**), morphine *N*-oxide (**3**) and 10-*S*-hydroxymorphine (**4**)

R_s of	Conditions[a]								
	A	B	C	D	E	F	G	H	I
1, **2**	18.2	18.3	19.4	20.9	22.2	1.0	18.8	0.0	24.2
1, **4**	8.2	7.8	10.3	6.1	6.3	6.4	7.5	3.8	3.7
3, **4**	1.7	1.0	4.4	7.0	8.1	13.1	11.8	12.4	6.2

[a] A, CE-1, pH 2.60; B, CE-1, pH 3.20; C, CE-2, pH 3.20; D, CE-1, pH 2.60, 10 mM β-CD; E, CE-2, pH 3.20, 10 mM β-CD; F, CE-1, pH 2.60, 10 mM γ-CD; G, CE-1, pH 2.60, 10 mM HP-β-CD; H, CE-1, pH 2.60, 10 mM HP-γ-CD; I, CE-1, pH 2.60, 10 mM DM-β-CD. CE-1, 56 cm × 50 mm, 30 kV, 100 mM Tris–phosphate buffer, 25°C; CE-2, 56 cm × 50 mm, PVA coated, 30 kV, 100 mM Tris–phosphate buffer, 25°C.

plain BGE due to different pK_a values of these compounds (8.05 and 7.13 for **1** and **3**, respectively [13]). Resolution was higher in PVA coated capillary than in the bare silica one (Table 1). Addition of cyclodextrins resolution diminished, the lowest ($R_s = 3.7$) was with DM-β-CD. Contrary to this finding there was the resolution of peaks of 10-*S*-hydroxymorphine and morphine *N*-oxide—very low in plain BGE in bare silica, highest in presence of γ-CD. Morphinane alkaloids are oxidized with hydrogen peroxide to two epimeric *N*-oxides [14]. Because the concentration of the minor *N*-oxide (with axially oriented *N*-methyl group) did not exceed 8% in synthesized *N*-oxides of morphine we considered only the major one in the further study. Separation of diastereomers of morphinane *N*-oxides will be published later.

Resolution of peaks of studied compounds was dependent on the concentration of added selector (Fig. 2). Further, we used 5 mM DM-β-CD in BGE as a compromise between the analysis time and resolution of peaks (Fig. 3).

Separation by CZE depends on difference in analytes velocities in an electric field; ion mobility in a capillary with constant length is a function of applied voltage. While the resolution of peaks of morphine and 10-*S*-morphine was proportional to applied voltage, resolution of peaks of internal standard and pseudomorphine as well as peaks of compounds **3** and **4** was only slightly affected by electric field. (Fig. 4).

Temperature of the capillary is an another factor affecting the mobility of ions and resolution of peaks. Mobility of ions increases with increasing temperature due to lower viscosity of BGE, on the other hand, temperature affects the dispersion and causes broadening of zones and consequently alters resolution. It is remarkable, that the resolution of peaks of morphine and 10-*S*-hydroxymorphine (**4**) increased with temperature, while the resolution of peaks of other compounds decreased (Fig. 5) as a consequence of dispersion effects.

Because concentration of degradation products in properly stored alkaloid substances or preparations should be low, about 0.2%, we used pyridoxine as an internal standard for quantification of morphine oxidation products.

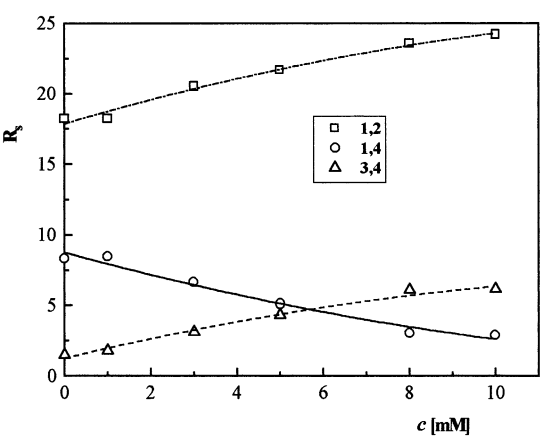

Fig. 2. Dependence of resolution of peaks of morphine (**1**), pseudomorphine (**2**) morphine *N*-oxide (**3**), and 10-*S*-morphine (**4**) on concentration of DM-β-CDX. Capillary: 56 cm × 50 mm, 100 mM Tris–phosphate, pH 2.60, 30 kV, 25°C.

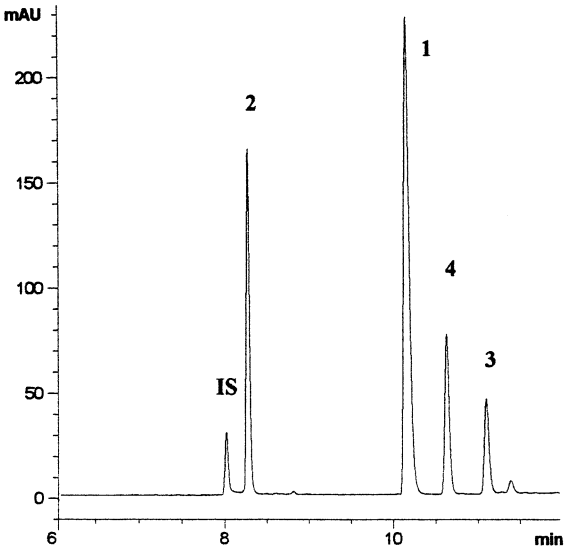

Fig. 3. Electropherogram of solution containing morphine (**1**) and its oxidation products: pseudomorphine (**2**), morphine *N*-oxide (**3**), 10-*S*-hydroxymorphine (**4**), pyridoxine (IS). Capillary: 56 cm × 50 mm, 100 mM Tris–phosphate pH 2.60, 5 mM DM-β-CDX, 30 kV, 25°C.

Injected volume of sample solution should be low in order to maintain high efficiency, however, the injection plug length is a more critical parameter than volume. The sample plug should be less than 2% of the total length of capillary, what is about 6.4 nl for 64.5 cm long capillary. Dependence of peak area on sample volume was linear in the range 1–15 nl ($r^2 = 0.999$, $P = 0.001$) for injected solution of pyridoxine (25 mg ml^{-1}), pseudomorphine (12.8 mg ml^{-1}), or morphine (0.5 mg ml^{-1}).

The detection limit of separated compounds was 0.8–1.3 mg ml^{-1}, calibration curve, determined by analysis at ten concentration levels was linear in the range 0.005–0.5 mg ml^{-1} for all studied compounds with regression coefficient better than 0.99, e.g. for pseudomorphine $c = 23.7x + 0.5$ ($x = A_x/A_{is}$, A_x, A_{is} = peak area of determined compound and reference substance, respectively) with $r^2 = 0.999$, $P = 0.001$.

The precision expressed by the repeatability has been determined by analysis of three samples (five injections) of prepared solutions of morphine and its oxidation products with pyridoxine as an internal standard with results (% RSD) as follows: migration time 0.30, 0.34, 0.33%, resolution of peaks 1.8, 1.3%, ratio of peak area versus area of IS 1.78, 1.10, 1.15% for morphine, 10-*S*-hydroxymorphine and morphine *N*-oxide, respectively. An electropherogram of sample containing pseudomorphine (0.13%), morphine *N*-oxide (0.09%) and 10-*S*-hydroxymorphine (0.21%) in morphine is shown in Fig. 6.

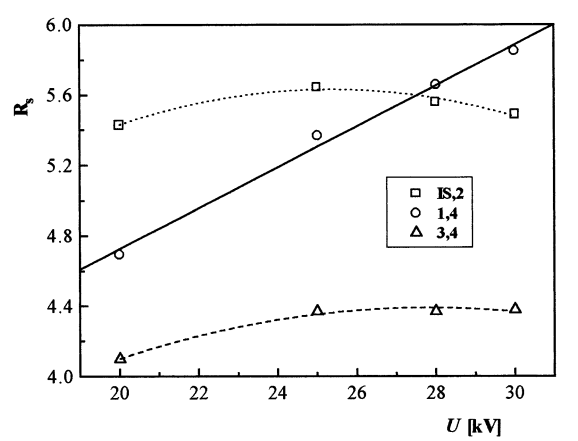

Fig. 4. Resolution of peaks of pyridoxine (IS), morphine (**1**), pseudomorphine (**2**), morphine *N*-oxide (**3**), and 10-*S*-hydroxymorphine (**4**) as a function of applied voltage. Capillary: 56 cm × 50 mm, 100 mM Tris–phosphate 5 mM DM-β-CDX, pH 2.60, 25°C.

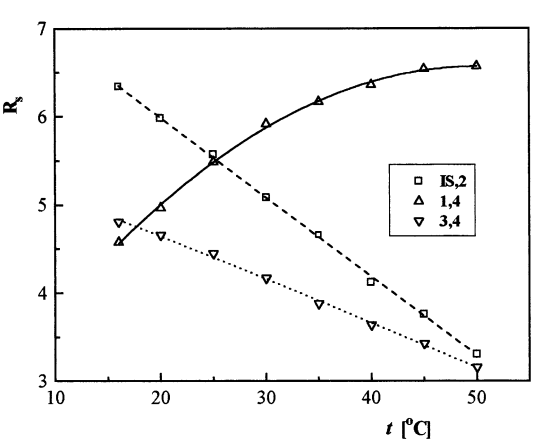

Fig. 5. Dependence of peak resolution of pyridoxine (IS), morphine (**1**), pseudomorphine (**2**), morphine *N*-oxide (**3**) 10-*S*-hydroxymorphine (**4**) on temperature. Capillary: 56 cm × 50 mm, 100 mM Tris–phosphate 5 mM DM-β-CDX, pH 2.60, 30 kV.

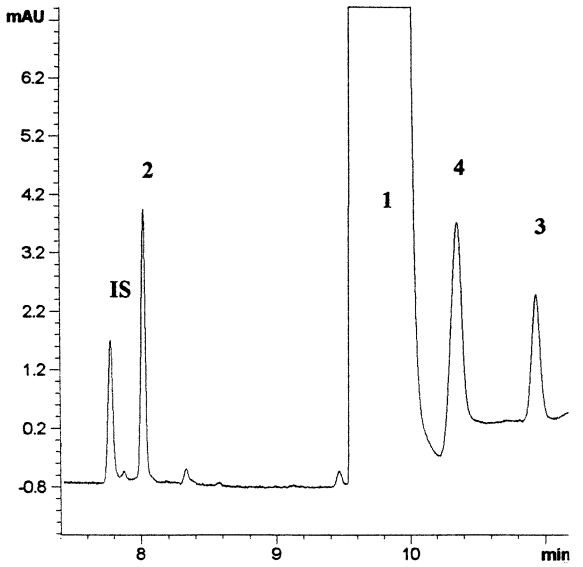

Fig. 6. Electropherogram of sample containing morphine (**1**, 99.26%), pseudomorphine (**2**, 0.13%), morphine *N*-oxide (**3**, 0.09%) and 10-*S*-hydroxymorphine (**4**, 0.21%). Capillary: 56 cm × 50 mm, 100 mM Tris–phosphate 5 mM DM-β-CDX, pH 2.60, 25°C, 30 kV.

4. Conclusions

Morphine and its oxidation products were separated by capillary zone electrophoresis in Tris–phosphate buffer. Completion of BGE with cyclodextrins substantially changed the selectivity; the highest resolution of all studied compounds was observed with β-CD and its derivatives. Pyridoxine proved to be a reliable internal standard for quantification of morphine oxidation products at their concentration below 0.5% in stored crystalline morphine or its salts.

References

[1] S.-Y. Yeh, J.L. Lach, J. Pharm. Sci. 50 (1961) 30–34.
[2] S.-Y. Yeh, J.L. Lach, J. Pharm. Sci. 50 (1961) 35–42.
[3] F.S. Eiger, J. Lameh, A. Rezvani, B.W. Gibson, W. Sadee, Pharm. Res. 7 (1990) 1205–1207.
[4] B. Proksa, Z. Votický, L. Molnár, J. Putek, M. Šteffek, Chem. Papers 32 (1978) 710–714.
[5] I. Bjørnsdottir, S.H. Hansen, J. Pharm. Biomed. Anal. 15 (1997) 1083–1089.
[6] I. Bjørnsdottir, S.H. Hansen, J. Pharm. Biomed. Anal. 13 (1995) 687–693.
[7] C.-X. Zhang, W. Thormann, J. Chromatogr. A 764 (1997) 157–168.
[8] F. Tagliaro, F.P. Smith, S. Turrina, V. Equisetto, M. Marigo, J. Chromatogr. A 735 (1996) 227–235.
[9] M.R. Taylor, S.A. Westwood, D. Perrett, J. Chromatogr. A 745 (1996) 155–163.
[10] T. Hyötyläinen, H. Sirén, M-L. Riekkola, J. Chromatogr. A 735 (1996) 439–447.
[11] K.W. Bentley, S.F. Dyke, J. Chem. Soc. (1959) 2574–2577.
[12] J.C. Craig, K.K. Purushothaman, J. Org. Chem. 35 (1970) 1721–1723.
[13] H. Rapoport, S. Masamune, J. Am. Chem. Soc. 77 (1955) 6359–6361.
[14] J.D. Phillipson, S.S. Handa, S.W. El-Dabbas, Phytochemistry 15 (1976) 1297–1299.