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Short communication

Separation of enantiomers of butorphanol and cycloamine by capillary zone electrophoresis

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Abstract

Butorphanol tartrate is a synthetic opioid agonist-antagonist used as analgesic, possessing three chiral centres in the basic part of the molecule. Its chiral purity is routinely controlled only by optical rotation. A new capillary zone electrophoresis method, capable to separate the enantiomers of butorphanol and intermediate of its synthesis, cycloamine, was developed. Different electrolyte composition (type and concentration of carrier ion, pH, and organic solvent addition), and type and concentration of several chiral selectors (natural and modified cyclodextrins) were tested. Using the optimized conditions (acidic electrolyte with the addition of highly sulphated gamma-cyclodextrin) as low as 0.05% of undesirable enantiomers can be detected. Selected method characteristics, i.e., linearity (0–50 mg/l), precision (2.5% at 20 mg/l), and accuracy (101 ± 2% at 20 mg/l) were evaluated. The optimized method was applied for the analysis of real batches of butorphanol and cycloamine. It was found that butorphanol tartrate manufactured by IVAX Pharmaceuticals contains less than 0.05% of undesirable enantiomer.

Keywords: Butorphanol; Cycloamine; Capillary electrophoresis; Chiral separation

1. Introduction

Butorphanol tartrate, chemically (−)-17-(cyclobutylmethyl)morphinan-3,14-diol D-(−)-tartrate (1:1) (salt), is a synthetic opioid agonist-antagonist used as analgesic: e.g., Stadol for human and Torbugesic and Torbutrol for veterinary use [1]. Butorphanol, possessing three chiral centres, was first time synthesized by multi-step total synthesis from 7-methoxytetralone in racemic form. The required, biologically active enantiomer with configuration 9R,13S,14R, further (−)-butorphanol, was obtained by resolution with D-(−)-tartaric acid [2]. Later a stereoselective synthesis from a commercially available chiral precursor (+)-2(4-methoxybenzyl)-1,2,3,4,5,6,7,8-octahydroisoquinoline was developed [3]. While substantial attention was devoted to the chemical and polymorphic purity of butorphanol tartrate, the chiral purity of the product was tested only by optical rotation [4,5]. Applications of electromigration techniques in chiral analysis one can find elsewhere [6,7]. On the other hand in currently accessible databases (Science Direct, Web of Science) we did not find any paper dealing with enantiomer separation of butorphanol or cycloamine.

In this work a capillary zone electrophoresis (CZE) separation of the enantiomers of butorphanol and an intermediate of its synthesis, called cycloamine (Fig. 1) has been studied and a method suitable for determination of the undesirable enantiomers of butorphanol and cycloamine has been developed. The basic characteristics, i.e., repeatability, accuracy, linearity and limit of detection of the developed CZE method were evaluated. The method was applied for enantiomeric purity testing of commercial substance of butorphanol tartrate.

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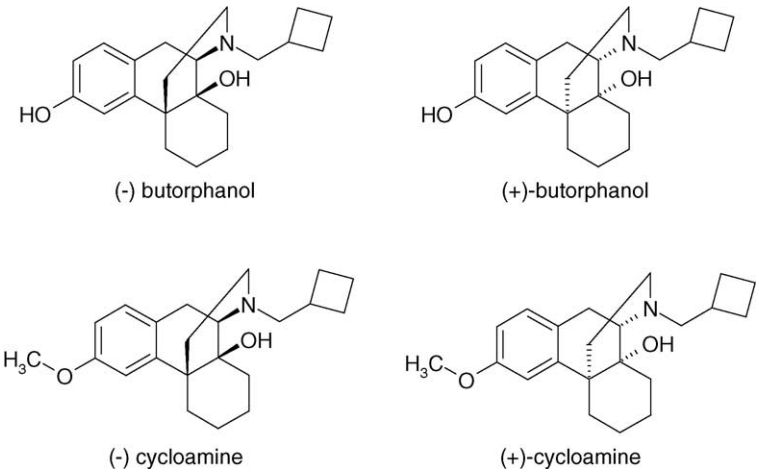

Fig. 1. Structures of butorphanol and cycloamine enantiomers.

2. Experimental

2.1. Chemicals

Standards of (+)- and (−)-butorphanol base, (+)- and (−)-cycloamine base and samples of commercial batches of butorphanol tartrate and cycloamine were obtained from IVAX Pharmaceuticals (Czech Republic). Phosphoric acid was purchased from Lachema (Czech Republic), α-cyclodextrin was obtained from CycloLab (Hungary), 1,1,1-tris(hydroxymethyl)aminomethane (Tris), β- and γ-cyclodextrin were obtained from Sigma–Aldrich (Czech Republic), and 20% (w/v) solution of highly sulphated gamma-cyclodextrin (HS-γ-CD) was purchased from Beckman Coulter (USA).

2.2. Instrumentation

CZE analyses were done with an electrophoretic analyser ^{3D}CE (Hewlett-Packard, USA) equipped with diode array detection (DAD).

2.3. Conditions of analysis

The CZE separation of butorphanol and cycloamine enantiomers was made in hydrodynamically open system (open tubular capillary). Analysis was performed in a fused silica capillary of 30 cm (23 cm effective length) ×50 μm i.d. (30 °C) and constant voltage applied to capillary was +10 kV (standard polarity setting, i.e., positive electrode is at the inlet vial). Optimized background electrolyte (BGE) consisted of 250 mM Tris + 350 mM H_3PO_4 + 0.02% HS-γ-CD. Samples were injected by pressure (25 mbar for 2 s) and separated analytes were detected at 200 nm. One analysis took 15 min. Internal standard method (five concentration levels of (+)-butorphanol and (+)-cycloamine-2, 4, 10, 20 and 40 mg/l). Adenine served as internal standard at concentration of 1 g/l. Standard and samples were dissolved in 0.1 M hydrochloric acid.

3. Results and discussion

Because of both butorphanol and cycloamine are weak bases (potentially bearing +1 charge) we use strong acidic background electrolyte for their analysis. Different carrier ions (sodium, lithium, BisTris propane and Tris) buffered with phosphoric acid (pH range 2–4) were tested. We found out that the electrolyte consisted of 250 mM Tris + 350 mM-H_3PO_4 is the best suited electrolyte for our purpose. In this electrolyte the separation of (±)-butorphanol and (±)-cycloamine was easily done within 10 min. To achieve enantiomer separation of butorphanol and cycloamine we tested several chiral selectors based on cyclodextrin. We found that the addition of α-cyclodextrin and β-cyclodextrin (5–20 mM) into background electrolyte slightly prolonged migration times of analytes but has practically no effect on enantiomer separation. It is due to fact that the cavity of α-cyclodextrin or β-cyclodextrin is too small for butorphanol and/or cycloamine molecules. On the other hand γ-cyclodextrin with wider cavity enabled enantiomeric separation. The 10 mM-γ-CD enabled baseline separation of butorphanol and cycloamine enantiomer. From the analyses it is clear that γ-CD influenced stronger on undesired (+) enantiomers than that of (−) ones. It is not favourable migration order from the point of view of enantiomeric purity testing when minor undesired enantiomer migrates at the tail of major one. That is why we looked for another selector and found out that highly sulphated γ-CD (HS- γ-CD) added to the background electrolyte gave inverse order of enantiomer migration (see Fig. 4). Furthermore, HS- γ-CD has stronger effect on chiral separation than that of natural γ-CD and this fact enabled to use lower concentration of HS-γ-CD (approximately 10 times lower). The stronger effect of HS- γ-CD can be explained by the negative charge of

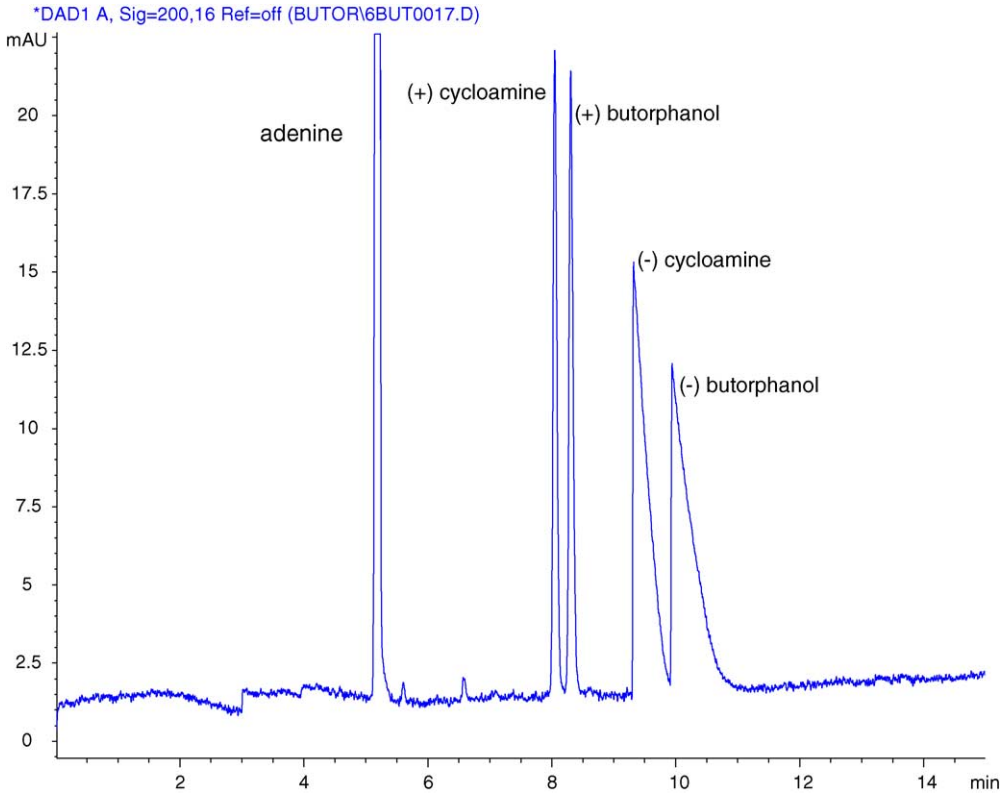

Fig. 2. Electropherograms of model mixture of (+/−)-butorphanol and (+/−)-cycloamine, each 0.05 g/l; background electrolyte: 250 mM-TRIS + 350 mM-phosphoric acid + 0.02% HS-γ-CD.

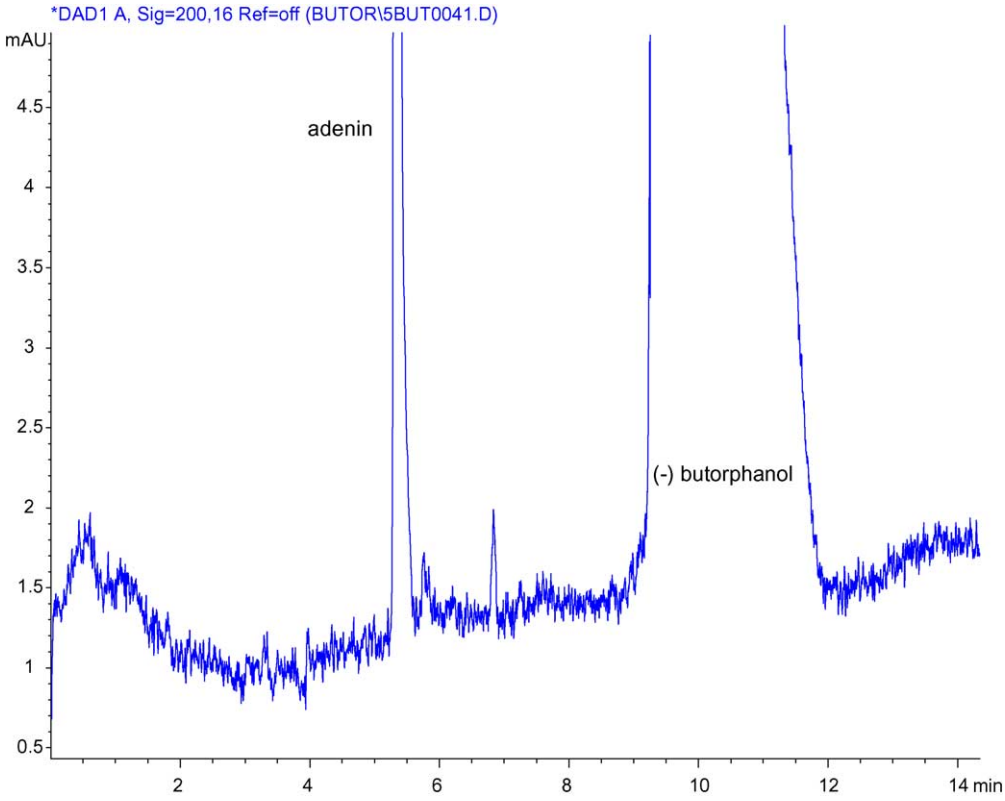

Fig. 3. Electropherograms of commercial (−)-butorphanol tartrate (2 g/l); analysis conditions—see Fig. 2.

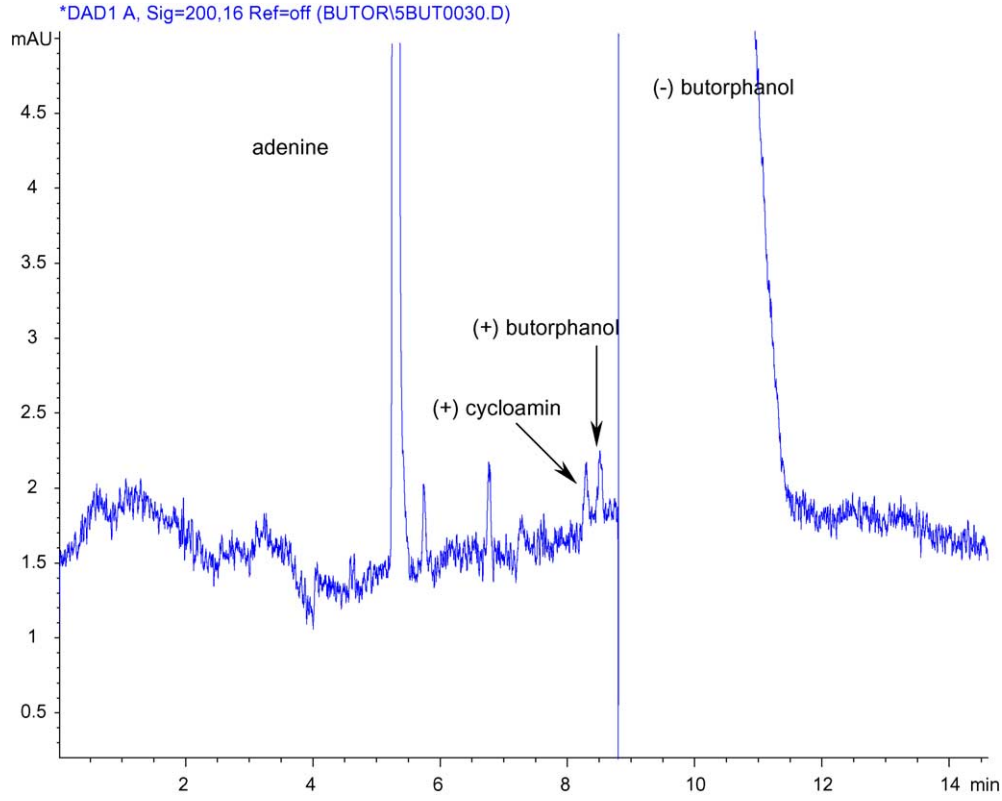

Fig. 4. Electropherograms of commercial (−)-butorphanol tartrate (2 g/l) spiked with (+)-butorphanol and (+)-cycloamine (0.002 g each/l); analysis conditions—see Fig. 2.

HS- γ-CD due to $-SO_3^-$ groups in its molecule (typical 7–10 sulphogroups per one molecule of γ-CD). Thus, HS- γ-CD migrates against the analytes resulting in stronger influence. This effect is visible from the peak shape of (−) enantiomers (see Fig. 2).

We studied the impact of HS- γ-CD concentration on migration time of analytes. Chiral selector was added into BGE at five levels (0.005, 0.01, 0.02, 0.05 and 0.1% w/v). From the results is evident that (−) enantiomers are stronger influenced than (+) enantiomers. At the concentration 0.1% (w/v) of HS- γ-CD the migration time of (−)-butorphanol and/or (−)-cycloamine is greater than 30 min and very broad peak is generate (data not shown). The addition of 0.02% of HS-γ-CD giving the baseline separation of enantiomers seemed to be an optimal (see Fig. 2).

The basic characteristics of the CZE method, i.e., linearity, precision, accuracy (recovery) and quantification limit are summarised in Table 1. Results clearly showed that the CZE method fulfils the pharmacopoeia criteria[1] and therefore it is suitable for intended purpose.

The optimized BGE was used for enantiomeric purity checking of commercial butorphanol substance (see Fig. 3). Under these conditions the undesired (+) enantiomer of butorphanol and (+)-cycloamine at 0.1% level could be quantified (see Fig. 4). In all analysed commercial batches of (−)-butorphanol and (−)-cycloamine the undesired (+) enantiomers were under detection limit, i.e., 0.05% (w/w).

Table 1
Method characteristics for CZE analysis of (+)-butorphanol and (+)-cycloamine

Characteristic	Value
Linearity (mg/l)[a]	0–50
Precision (RSD in % of 20 mg/l, $n = 6$)	2.50
Accuracy (recovery in % at 2–40 mg/l addition, $n = 5$)	101 ± 2
LOD (S/N = 3, mg/l)	1[b]

[a] Correlation coefficient for (+)-butorphanol and (+)-cycloamine is 0.9995 and 0.9993, respectively.
[b] Corresponds to 0.05%.

4. Conclusion

The developed electrophoretic method is suitable for the checking of enantiomeric purity of butorphanol tartrate. It enables detection as low as 0.05% of undesired (+) enantiomers of butorphanol and/or cycloamine in the active substance. The basic characteristics of the CZE fulfil general validation criteria and thus the method is suitable for intended purpose.

Results of analyses of real samples showed that the commercial active substance of butorphanol tartrate manu-

[1] LOD of undesirable enantiomer should be as low as 0.1%.

factured by IVAX Pharmaceuticals (Czech Republic) contains less than 0.05% of the undesired enantiomer of butorphanol.

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