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Short communication

Capillary zone electrophoresis separation of enantiomers of lisuride

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Abstract

Lisuride is an ergot alkaloid derivative with dopaminergic activity. It is used for treatment of Parkinsonism and some other diseases associated with high level of prolactine. Lisuride is a chiral compound derived from natural ergot alkaloids. A new capillary zone electrophoresis (CZE) method capable of separating the enantiomers of lisuride was developed. Using the optimized conditions (acidic electrolyte with the addition of gamma-cyclodextrin (γ-CD)) as low as 0.02% of undesirable L-lisuride can be detected. Selected method characteristics, i.e., linearity (0–20 mg/l), precision (2.0% at 5 mg/l), and accuracy (101 ± 4% at 5 mg/l) were evaluated. The optimized method was applied for the analysis of real batches of Lisuride hydrogenmaleate and Lisuride base manufactured by IVAX Pharmaceuticals. It was found that they contain less than 0.02% of undesirable L-enantiomer.

Keywords: Lisuride; Capillary electrophoresis; Chiral separation

1. Introduction

Lisuride (Fig. 1) is a semi-synthetic ergot alkaloid with dopaminergic activity [1,2]. In the form of a salt with maleic acid (Lisuride hydrogenmaleate) it is used as inhibitor of prolactine secretion and as antiparkinsonian drug (Dopergin®, Cuvalit®). While the chiral purity of natural ergot alkaloids (e.g., ergocristine) is assured by their biosynthesis (they are derived from L-tryptophan) and their racemization is not probable (ergocristine contains six chiral centers), the chiral purity of lisuride is not so evident. It contains only two chiral centers and although it is manufactured from natural ergocristine [3], racemization during the synthetic steps should be anticipated. Therefore, some control of chiral purity of lisuride is required. For the time being, only optical rotation is involved. A simple separation method would be very desirable for routine analysis of chiral purity of lisuride.

HPLC separation of D- and L-lisuride was achieved on a new silica-based stationary phase, with covalently bounded chiral selector. The used chiral selector was in fact a derivative of lisuride (1-(3-aminopropyl)-*trans*-dihydrolisuride) [4]. Because of the chiral phase is not commercially available, the method is not generally applicable. Applications of electromigration techniques in chiral analysis one can find elsewhere [5,6]. Separation of lisuride enantiomer by capillary zone electrophoresis (CZE) was published by Fanali et al. [7].

Lisuride enantiomers were separated in acidic 0.1 M sodium phosphate buffer with gamma-cyclodextrin (γ-CD) as chiral selector. Maximum resolution ($R = 1.1$) of lisuride enantiomers was reached at 80 mM γ-CD. In the case of enantiomeric purity check analysis at 0.1% levels of undesired enantiomer such resolution is not sufficient.

In this work, an electrophoretic separation of the enantiomers of lisuride (Fig. 1) has been studied and method suitable for the determination of the undesirable L-enantiomer of lisuride 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 Lisuride hydrogenmaleate and Lisuride base.

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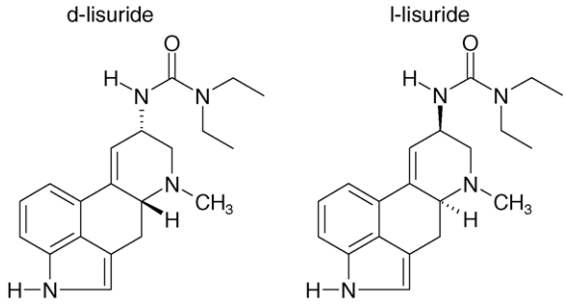

Fig. 1. Structures of lisuride enantiomers.

2. Experimental

2.1. Chemicals

Standards of D- and L-lisuride and samples of commercial batches of lisuride maleate (seven batches) and Lisuride base (five batches) were obtained from IVAX Pharmaceuticals (Opava, Czech Republic). Phosphoric acid was purchased from Lachema (Brno, Czech Republic), 1,1,1-tris-hydroxymethylaminomethane (Tris) and γ-CD were obtained from Sigma-Aldrich (Prague, Czech Republic).

2.2. Instrumentation

CZE analyses were done with an electrophoretic analyzer Hewlett Packard^{3D} CE (HPST Ltd., Prague, Czech Republic) equipped with diode array detector (DAD).

2.3. Conditions of analysis

The CZE separation of lisuride enantiomers was performed in a fused silica capillary having a total length 350 mm (265 mm effective length, 50 μm i.d.) and constant voltage applied to capillary was +12 kV. Optimized background electrolyte (BGE) consisted of 100 mM Tris + 140 mM H_3PO_4 + 20 mM γ-CD. Samples were injected by pressure (25 mbar for 5 s) and separated analytes were detected at 230 nm. The analysis time was 15 min. Internal standard method (five concentration levels of L-lisuride—1, 2, 5, 10, and 20 mg/l) was used. Adenine served as internal standard at concentration of 0.25 g/l. Standard and samples were dissolved in 0.01 M HCl. Sample concen-

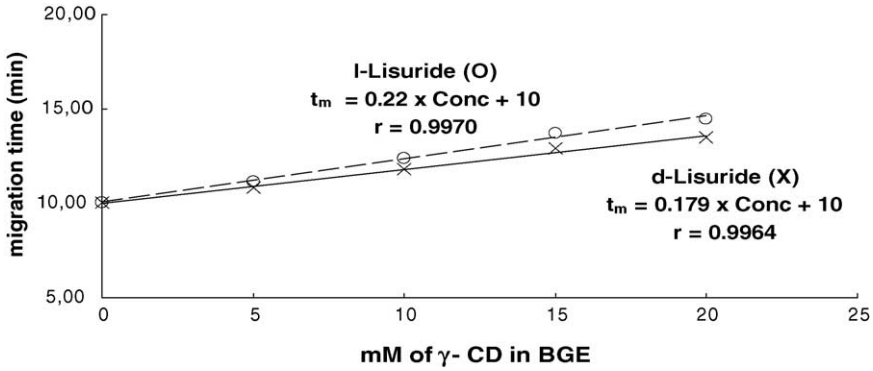

Fig. 2. Effect of γ-CD addition into BGE on separation of D- and L-lisuride.

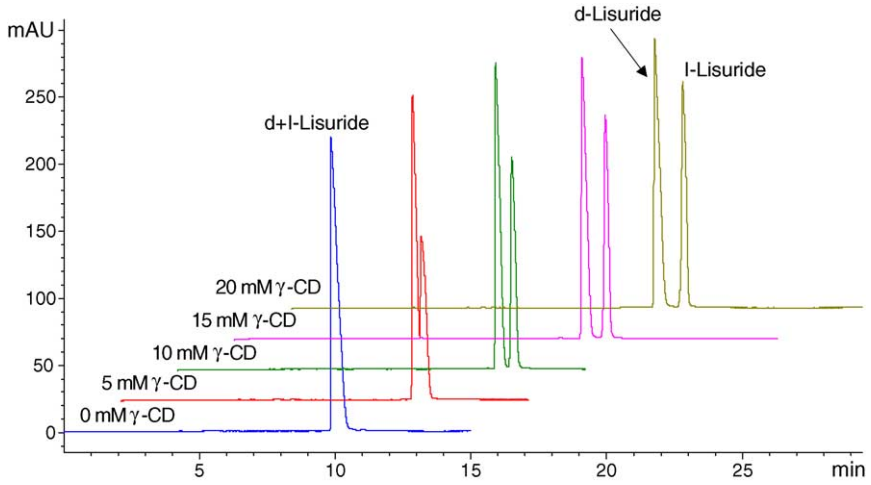

Fig. 3. Electropherograms of model mixture of D-lisuride (0.6 g/1) and L-lisuride (0.4 g/1); background electrolyte: 100 mM Tris + 140 mM phosphoric acid + 0–20 mM γ-CD.

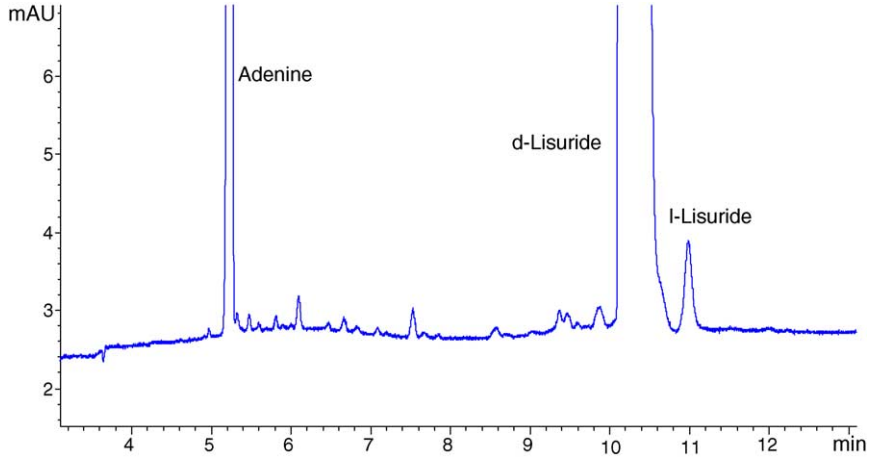

Fig. 4. Electropherograms of commercial D-lisuride maleate (1 g/1) spiked with L-lisuride (2 mg/1); background electrolyte: 100 mM Tris + 140 mM phosphoric acid + 20 mM γ-CD.

tration of commercial substance of lisuride was 1 g/l of 0.01 M HCl.

3. Results and discussion

Because of lisuride is a weak base (potentially bearing +3 charges) it is fully protonated at low pH. That is why we use acidic background electrolyte for its analysis. As a carrier ion we applied organic base Tris buffered with phosphoric acid in pH range 2–4. We found out that the electrolyte consisted of 100 mM Tris +140 mM H_3PO_4 (pH 2.5) is the best-suited electrolyte for our purpose. To achieve enantiomer separation of lisuride, we tested several chiral selectors based on cyclodextrin. The addition of α-cyclodextrin and β-cyclodextrin (5–20 mM) into background electrolyte caused slight prolongation of migration times of analytes but has no effect on enantiomer separation. It is due to small cavity of α-cyclodextrin or β-cyclodextrin for lisuride molecule. On the other hand, γ-CD enabled enantiomeric separation. The impact of γ-CD on migration time of lisuride enantiomers is depicted in Figs. 2 and 3. From these results it is clear that under given condition L-lisuride forms stronger inclusion complex with γ-CD than that of D-lisuride. Within the used concentration range of chiral selector each mmol of γ-CD added into BGE causes approximately 0.18 min increment of migration time (t_m) of D-lisuride of while for L-lisuride of 0.22 min. The baseline separation ($R = 1.25$) of lisuride enantiomers was reached at the 10 mM concentration of γ-CD. Unfortunately minor undesired L-enantiomer migrates at the tail of major one. This is not favorable migration order from the point of view of enantiomeric purity

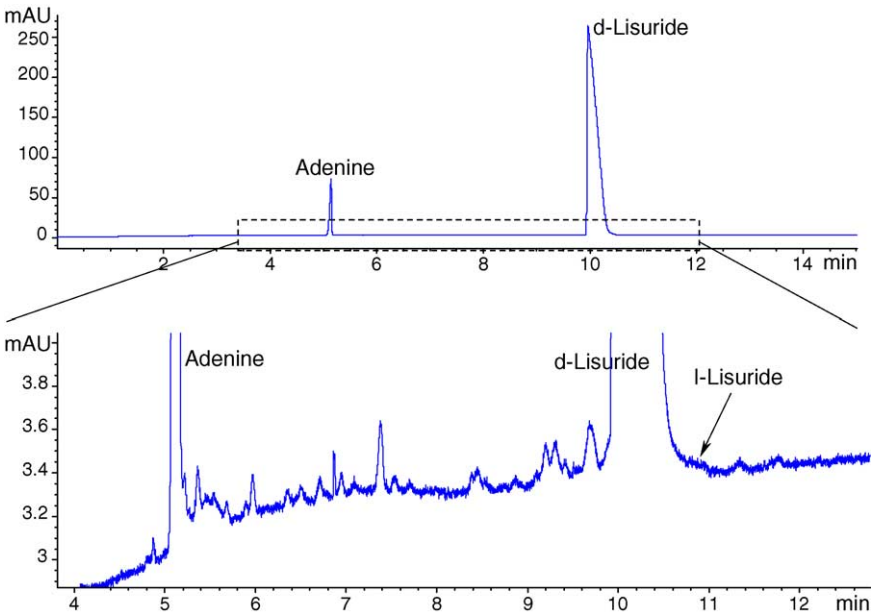

Fig. 5. Electropherograms of commercial Lisuride hydrogenmaleate (1 g/1); analysis conditions—see text.

Table 1
Method characteristics for CZE analysis of L-lisuride

Characteristic	Value
Linearity (mg/l)[a]	0–20
Precision (RSD in % of 5 mg/l, $n = 6$)	2.00
Accuracy (recovery in % at 1–20 mg/l addition, $n = 5$)	101 ± 4
LOD (signal/noise = 3, mg/l)	0.2[b]
LOQ (S/N = 10, mg/l)	0.5

[a] Correlation coefficient for L-lisuride was 0.9998.
[b] Corresponds to 0.02%.

testing when minor component migrates at the tail of major one. Due to this migration order we looked for another selector and we tried to use highly sulfated γ-cyclodextrin (HS–γ-CD). We found out that the migration order was the same as in case of γ-CD. Furthermore the peak shape was strongly asymmetric and very broad (data not shown). That is why we returned to γ-CD. To detect as low as 0.1% of L-lisuride we had to increase the concentration of γ-cyclodextrin up to 20 mM ($R = 1.74$). Electropherogram of D-lisuride spiked with 0.2% of L-enantiomer is depicted in Fig. 4.

The basic characteristics of the CZE method, i.e., linearity, precision, accuracy (recovery) and quantification limit are summarized in Table 1. Results clearly showed that 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 lisuride substance (see Fig. 5). Under these conditions the undesired L-enantiomer of lisuride at 0.05% level could be quantified. In all analyzed commercial batches of Lisuride base and/or Lisuride hydrogenmaleate the undesired L-enantiomer was under detection limit, i.e., 0.02% (w/w).

4. Conclusion

The developed electrophoretic method is suitable for the checking of enantiomeric purity of Lisuride hydrogenmaleate and Lisuride base. It enables detection as low as 0.02% of undesired L-enantiomer of lisuride in the active substance. The basic characteristics of the CZE fulfill general validation criteria and thus the method could be used in routine analysis. Results of analyses of real samples showed that the commercial active substance of both Lisuride hydrogenmaleate and Lisuride base manufactured by IVAX Pharmaceuticals (Opava, Czech Republic) contains less than 0.02% of the undesired L-lisuride.

Acknowledgement

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[1] LOD of undesired enantiomer should be 0.1% of enantiomer.