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Journal of Chromatography A, 731 (1996) 315–321

JOURNAL OF CHROMATOGRAPHY A

Short communication

Determination of enantiomerization barriers by computer simulation of experimental elution profiles obtained by high-performance liquid chromatography on a chiral stationary phase

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Received 30 June 1995; revised 26 October 1995; accepted 26 October 1995

Abstract

The enantiomers of oxazepam, chlorthalidone and phenylthiohydantoin-phenylalanine (PTH-phenylalanine) were separated by HPLC using chemically bonded β-cyclodextrin (ChiraDex) as chiral stationary phase. The obtained chromatograms showed typical plateaus between the peaks of the separated enantiomers, indicating enantiomerization, i.e. reversible enantiomer interconversion. Computer simulation of the experimental chromatographic elution profiles was employed for the determination of rate constants and corresponding enantiomerization barriers for the above compounds. The data were compatible with independently determined values, but a very strong dependence on the solvent was observed.

Keywords: Computer simulation; Elution profiles; Enantiomerization; Racemization; Chiral discrimination; Oxazepam; Chlorothalidone; PTH-Phenylalanine

1. Introduction

It is well known that the biological activity of many drugs is strongly related to chirality. Thus, frequently only one of the enantiomers shows the desired therapeutic effect while the other one with opposite configuration is inactive or shows undesirable side effects [1]. Therefore, it is important to study the configurational stability of those drugs that are administered as pure enantiomers.

Two processes have to be distinguished when the configurational or conformational lability of chiral compound results in an interconversion of its enantiomers. Enantiomerization at the microscopic and molecular level is the reversible interconversion of the enantiomers [2], i.e. $(+) \rightleftarrows (-)$ (rate constant $k_1 = k_{-1}$). Racemization at the macroscopic and statistical level, on the other hand, is often considered the irreversible transformation of an enantiomerically enriched or pure form into the racemic mixture, i.e. $(+) \rightarrow (\pm)$, (rate constant k_2). It is noted that $k_2 = 2k_1$, because the inversion of one molecule reduces the optical purity by two molecules [3].

The phenomenon of enantiomerization can easily be studied by dynamic chromatographic methods as predicted [4] and exemplified in GC [5–8] and HPLC [9–11]. If enantiomerization proceeds at a suitable rate on the chromatographic time scale a

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0021-9673/96/$15.00
SSDI 0021-9673(95)01230-3

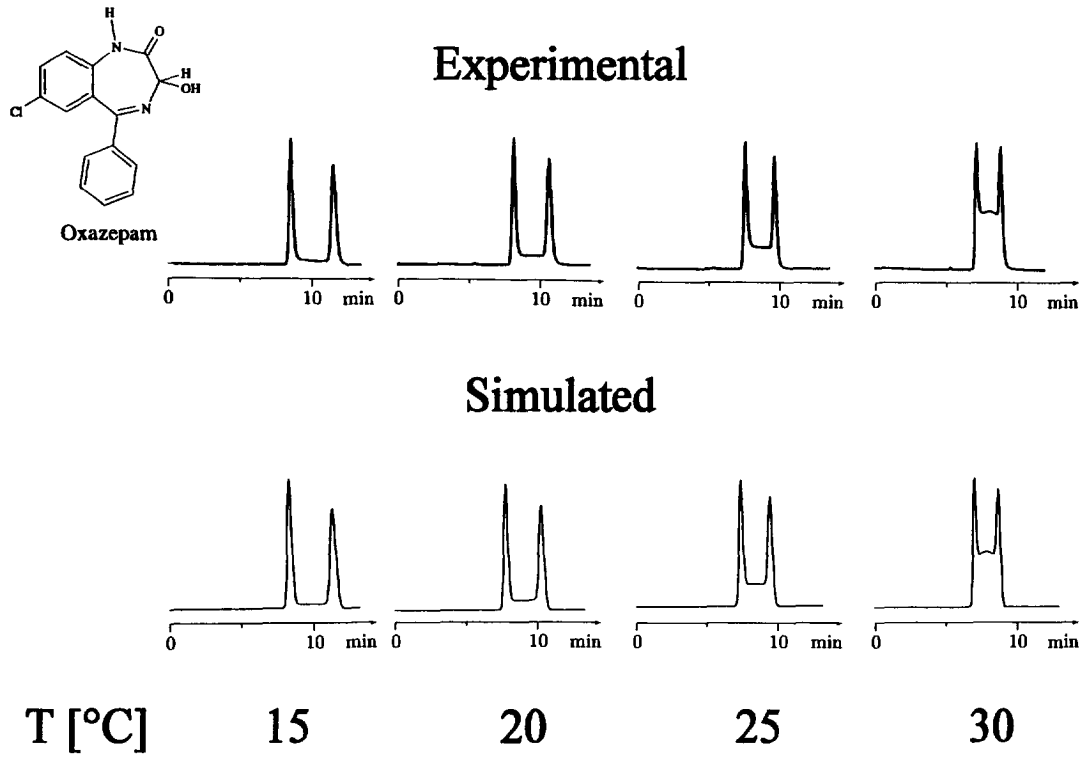

Fig. 1. Experimental and simulated chromatograms of oxazepam at different temperatures. Column: ChiraDex, 250 × 4 mm I.D.; mobile phase: methanol–0.01 *M* phosphate buffer (pH 2.8) (40:60, v/v), 0.5 ml/min.

typical plateau between the peaks of the two separated enantiomers can be observed in the elution profile. Furthermore, by peak form analysis using computer simulation the rate constants and energy barriers for enantiomerization can be determined.

Thus, Schurig and co-workers [5–7] used a computer program based on the discontinuous theoretical plate model for evaluation of elution profiles observed in GC, while Mannschreck and co-workers [9,10] and Veciana and Crespo [11] evaluated profiles observed in HPLC on the basis of a special Gauss type function for the elution curve (stochastic model).

This report deals with enantiomerization of oxazepam, chlorthalidone and phenylthiohydantoin-phenylalanine (PTH-phenylalanine). The enantiomers were separated by HPLC using chemically bonded β-cyclodextrin (ChiraDex) as chiral stationary phase. As far as oxazepam is concerned, Jira et al. [12] also obtained the typical plateau by chiral HPLC on Cyclobond, and, in addition, Wännman et al. [13] observed a pronounced plateau by micro-HPLC on a BSA-silica stationary phase, although their peaks were rather broad and tailed. More recently, Boonkerd et al. [14] showed the formation of a plateau in the elution profiles of enantiomers of 3-hydroxy-1,4-benzodiazepines in MEKC. In this work we demonstrate the applicability of our model and computer program [15] not only to GC, but also to HPLC elution profiles. The latter are more difficult to evaluate because of the more pronounced peak width and peak asymmetry. Rate constants and energy barriers for enantiomerization are determined by using the computer simulation program originally devised for GC [7].

2. Experimental

All solvents were of LiChrosolv grade (E. Merck,

Darmstadt, Germany). The HPLC column LichroCART, ChiraDex, 250 × 4 mm I.D., 5 μm (β-cyclodextrin chemically bonded to silica) is a commercially available product of Merck. The instrumentation comprises a HPLC system from Merck/Hitachi with a L-6200 intelligent pump, a L-4000 UV-detector, a D-2500 integrator and a Rheodyne injection valve. Temperature experiments were performed with an Inlabo F 10 UC thermostat. UV detection was performed at 220 nm.

Using the retention times and theoretical plate numbers measured from the experimental chromatograms, the simulation was performed as previously described in detail [7]. Briefly, an experimental chromatogram was simulated with a number of different rate constants k^m and k_1^s (the respective k_{-1}^s, is calculated from k_1^s according to the retention factors) until an optimal fit of simulated and experimental elution profiles was obtained. In contrast to Ref. [7], in the present case the rate constants of enantiomerization were assumed as equal in the mobile and the stationary phase. As a consequence, the hold-up time does not have any influence on the resulting simulated elution profiles.

The following data were used as a basis for the simulation (retention times for the enantiomer peaks in minutes, theoretical plate numbers of the enantiomer peaks):

Oxazepam [mobile phase: methanol–phosphate buffer (pH 2.8) (40:60, v/v)]: at 15°C: 8.37, 11.40; 1550, 2150; at 20°C: 7.89, 10.41; 1550, 2300; at 25°C: 7.48, 9.57; 1470, 2200; at 30°C: 7.13, 8.85; 1800, 2700; hold-up time: 5.22 min (all temperatures).

Chlorthalidone [mobile phase: methanol–water (20:80, v/v)]: at 6°C: 23.51, 37.60; 2200, 2200; at 10°C: 16.75, 25.54; 1900, 2100; at 15°C: 12.55, 18.31; 1750, 2000; hold-up time: 2.87 min (all temperatures).

Chlorthalidone [mobile phase: methanol–TEAA (pH 4.1) (2:98, v/v)]: at 45°C: 15.44, 20.67; 1750, 1650; at 50°C: 14.02, 18.12; 2000, 1800; at 55°C: 12.30 15.47; 1900, 1750; hold-up time: 3.46 min (all temperatures).

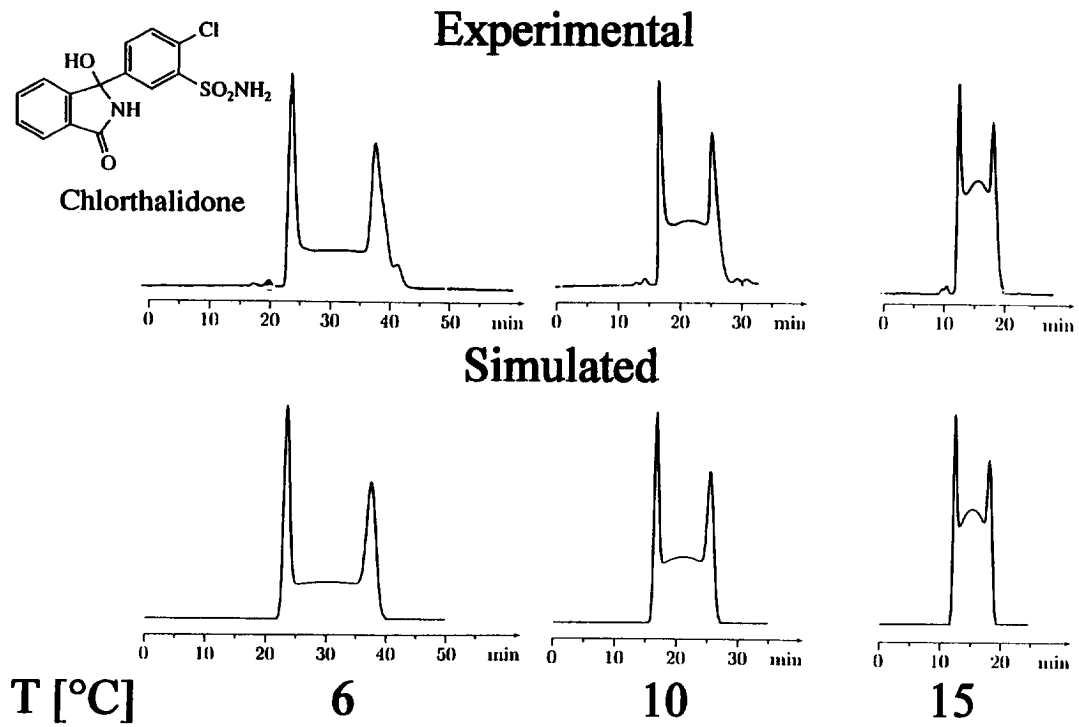

Fig. 2. Experimental and simulated chromatograms of chlorthalidione at different temperatures. Column: ChiraDex, 250 × 4 mm I.D.; mobile phase: methanol–water (20:80, v/v), 1 ml/min.

PTH-phenylalanine [mobile phase: methanol–TEAA (pH 4.1) (2:98, v/v)]: at 25°C: 21.06, 26.17; 3480, 4000; at 30°C: 18.56, 22.33; 2700, 2800; at 35°C: 16.34, 19.09, 2310, 2500; hold-up time 3.50 min (all temperatures).

3. Results and discussion

Two drugs, oxazepam and chlorthalidone, as well as an amino acid derivative, PTH-phenylalanine, were separated into their enantiomers by HPLC using silica-bonded β-cyclodextrin (ChiraDex) as chiral stationary phase. The obtained elution profiles showed typical plateaus between the two peaks of the separated enantiomers indicating that enantiomerization was taking place within the chromatographic time scale (Fig. 1, Fig. 2, Fig. 3, and Fig. 4). As expected, enantiomerization was more pronounced at higher temperatures.

Computer simulation using the experimental chromatographic elution profiles was performed according to a procedure described previously in detail [7]. The calculated forward and backward overall rate constants for enantiomerization, k_1, and k_{-1}, are given in Table 1. These rate constants are valid for the overall chromatographic system and include the contributions from the values for the mobile phase, k_1^m, and for the stationary phase, k_1^s and k_{-1}^s, respectively. The weight of these contributions is in turn determined by the residence times of the enantiomers in the respective phase, i.e. the retention factors. The calculated energy barriers $\Delta G^{\neq}$ for the enantiomerization of oxazepam, chlorthalidone and PTH-phenylalanine lie between 80–103 kJ/mol (cf. Table 1). These values are relatively low and confirm the configurational lability of the studied compounds.

The determined rate constants can be compared to rate constants obtained by independent methods, e.g. investigation of the racemization kinetics of optically enriched mixtures [16–18]. However, much care has to be exercised for two reasons. First, enantiomeriza-

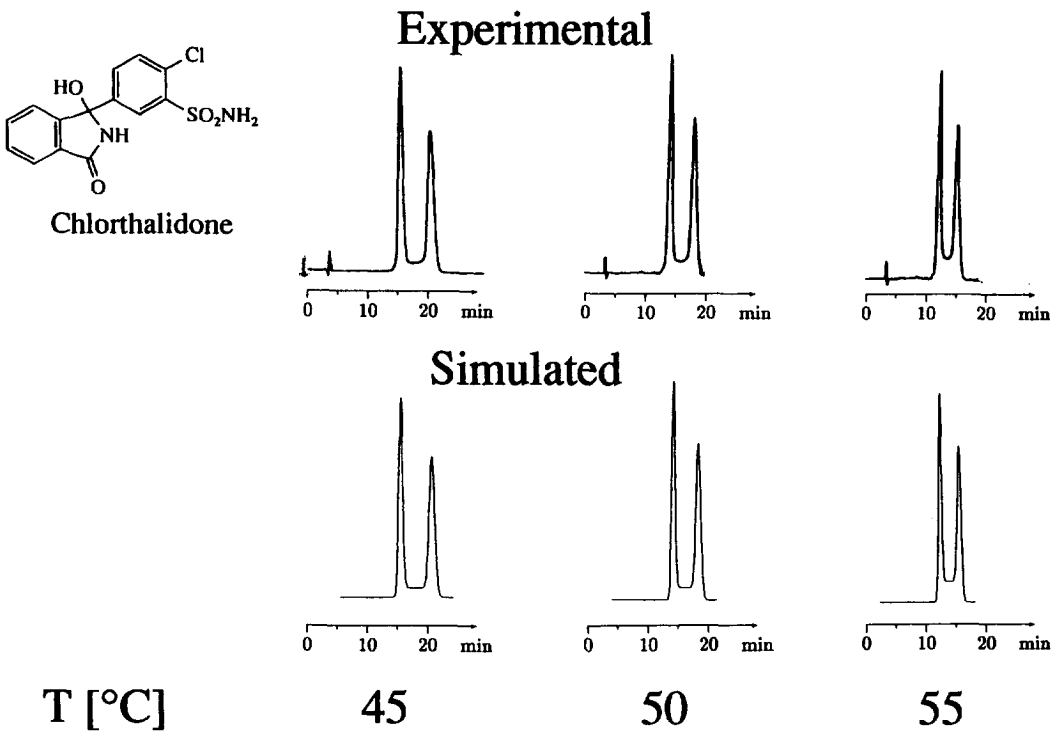

Fig. 3. Experimental and simulated chromatograms of chlorthalidone at different temperatures. Column: ChiraDex, 250 × 4 mm I.D.; mobile phase: methanol–triethylammonium acetate buffer (pH 4.1) (2:98, v/v), 0.8 ml/min.

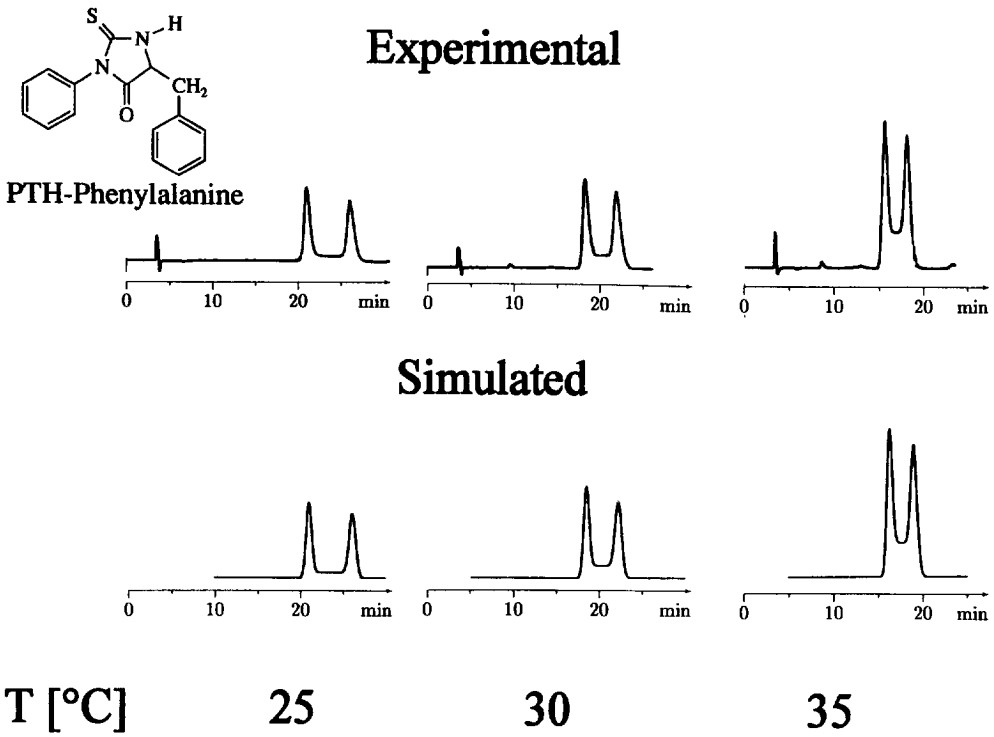

Fig. 4. Experimental and simulated chromatograms of PTH-phenylalanine at different temperatures. Column: ChiraDex, 250 × 4 mm I.D.; mobile phase: methanol–triethylammonium acetate buffer (pH 4.1) (2:98, v/v), 0.8 ml/min.

tion of oxazepam and chlorthalidone was found to depend very strongly on the solvent; and no data are available for the solvent mixtures used in our work. Second, there seems to be some confusion concerning the definition of the rate constant k; Aso et al. [16] quotes k for the reversible enantiomerization, i.e. (+)⇌(−), but throughout his work the term racemization is used. He calculates k from the half-life time $t_{1/2}$ according to the reversible first order kinetics, i.e. $t_{1/2}=\ln 2/(2k)$ (cf. kinetics textbooks, e.g. [19]). On the other hand, Yang [17,20] quotes k for irreversible racemization, i.e. (+)→(±) and evaluates according to irreversible first-order kinetics, i.e. $t_{1/2}=\ln 2/k$. In spite of this, in his introduction he uses Aso's enantiomerization k values for comparison with his racemization k values without correcting them by a factor of 2.

Aso et al. [16] demonstrated that the rate constant of the reversible enantiomerization in an aqueous system remains fairly constant between pH 2.4 and 9 at $k=0.0438$ min^{-1} (20°C). Yang et al. [17] determined half lives of oxazepam in various solvents by a spectropolarimetric method. According to $t_{1/2}=\ln 2/(2k)$ (cf. remarks above), their data correspond to $k=0.116$ min^{-1} (in an aqueous system, 23°C) and $k=0.072$ min^{-1} (in methanol, 23°C); much lower rates were obtained for less polar organic solvents. When extrapolating our data, which were determined in buffer–methanol (cf. Table 1), to the given conditions, we find them quite compatible with Aso's value for the aqueous system, whereas Yang's rate constants are higher by a factor of approx. 3. Further interpretation is difficult because of the extremely strong dependence on the solvent.

Severin [18] determined the rate constant of enantiomerization of chlorthalidone in water as 0.0065 and 0.0181 min^{-1} at 7 and 15°C, respectively. This is lower than our values (cf. Table 1, for methanol–water) by a factor of approx. 4, which might be explained by the presence of 20% methanol (v/v) in our experiments. The values determined for the more acidic eluent and higher temperatures (cf.

Table 1
Overall[a] rate constants of enantiomerization[b] k (min^{-1}) and the corresponding enantiomerization barriers[c] $\Delta G^{\neq}$ (kJ/mol) for three analytes on ChiraDex as stationary phase

T (°C)	k_1 (min^{-1})	k_{-1} (min^{-1})	$\Delta G^{\neq}$ (±0.5) (kJ/mol)
(a) Oxazepam			
15	0.0123	0.0091	89.5
20	0.027	0.020	89.2
25	0.054	0.042	89.0
30	0.114	0.092	88.6
(b) Chlorthalidone			
Mobile phase: methanol–water (20:80, v/v)			
6	0.032	0.020	84.6
10	0.059	0.038	84.4
15	0.104	0.070	84.5
Mobile phase methanol–TEAA (pH 4.1) (2:98, v/v)			
45	0.009	0.007	99.9
50	0.011	0.009	100.9
55	0.019	0.015	101.0
(c) PTH-phenylalanine			
25	0.0096	0.0077	93.2
30	0.0153	0.0127	93.6
35	0.023	0.019	94.2

[a] For the entire chromatographic system consisting of mobile and stationary phase (see text).
[b] For the entire interconversion of a molecule into the other enantiomer (cf. text); obtained by simulation and comparison to the experimental elution profiles (see Figs. 1–4, cf. Section 2).
[c] For the first, rate-determining step of enantiomerization, converting a molecule into the achiral intermediate (cf. text); calculated from the corresponding rate constants (average of k_1 and k_{-1}) according to the Eyring equation as previously described [7], using a transmission coefficient $f=0.5$ for the two-step reaction.

Table 1, right side) correspond more closely with the energy barrier determined by Severin for the reversible reaction of chlorthalidone to a dehydrated intermediate. Since Severin stated that there was no influence of pH in the range of 1 to 10, this might be a consequence of the composition of the eluent.

To the best of the authors' knowledge for PTH-phenylalanine no data are available in the literature.

As a consequence, our data are compatible with those obtained by other methods. The extremely strong dependence on the solvent does, however, not permit the exclusion of the possibility of any small to moderate activating or inhibitory effect of the cylodextrin in the stationary phase, as described for two diaziridines in dynamic GC [7]. However, at least for oxazepam, this effect cannot be great since similar plateaus were also obtained by HPLC on other stationary phases [12,13].

All three described enantiomerization equilibria proceed through achiral intermediates. According to reports by several authors [12,16,17], the enantiomerization of oxazepam is assumed to be due to a tautomeric equilibrium, via an achiral iminoaldehyde. Severin [18] proposed a planar carbenium ion as achiral intermediate for the enantiomerization of chlorothalidone. As a consequence of this two-step reaction mechanism, only half of the intermediate reacts to the other enantiomeric form, whereas the other half reacts back to the starting material. As described previously [7], this means that a transmission factor $f=0.5$ has to be used when calculating $\Delta G^{\neq}$ for the first step from the rate constants k of the entire process according to the Eyring equation (cf. Table 1).

4. Conclusion

Computer simulation of experimental dynamic chromatographic elution profiles is a simple and straightforward method for the determination of rate constants and energy barriers of enantiomerization phenomena. The method requires only trace amounts of the racemic sample and is suitable for barriers in the range of 70–130 kJ/mol, where dynamic NMR techniques fail to be applicable. However, in the described HPLC experiments, the strong dependence of the rate constants on the solvent used imposes severe limitations on the interpretation of the results. This means that the results are not universally valid, but are only applicable to the particular system of solvent and stationary phase. Our results are drastically affected by the choice of the mobile phase, and in addition, the stationary phase might also have a certain influence. A further systematic series of experiments will be required for a full understanding of the influence of solvent and pH.

Acknowledgments

V.S. thanks Deutsche Forschungsgemeinschaft and the Fonds der chemischen Industrie for continuous support of this work.

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