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DETERMINATION OF MEXILETINE IN BIOLOGICAL FLUIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatographic method for the determination of the antiarrhythmic drug mexiletine in human plasma, urine, and cerebrospinal fluid is described. Following extraction with diethyl ether, mexiletine and the internal standard 4-methylmexiletine were derivatized with 2,4-dinitrofluorobenzene. Analyses were performed using an alternating on-column enrichment technique on small Perisorb RP-2 30—40 μm pre-columns with pre-column backflushing for direct injection onto the analytical column of Spherisorb ODS 5 μm. Complete separation from endogenous constituents of plasma, urine or cerebrospinal fluid was achieved by isocratic reversed-phase ion-pair chromatography with 1-heptanesulfonic acid (0.005 *M*; PIC B7)—acetonitrile—tetrahydrofuran (42:48:10, v/v) as eluent. Interferences from other drugs were not observed. The limit of detection was 10 ng/ml (C.V. 6.5%). Day-to-day coefficients of variation were below 9%. The applicability of this rapid method for pharmacokinetic studies and clinical routine is demonstrated.

INTRODUCTION

Mexiletine (Mexitil^{R}) is an antiarrhythmic drug recently introduced for therapy and prophylaxis of ventricular arrhythmias [1—5]. Due to its oral efficacy and long plasma half-life (9—16 h) mexiletine compares favorably with currently available antiarrhythmic drugs such as lidocaine [6]. Intravenous or oral administration of mexiletine is followed by slow but extensive biotransformation to inactive metabolites [7, 8]. Therapeutic plasma concentrations of mexiletine have been reported to be 0.5—2.0 μg/ml, whereas toxicity becomes common above 3.0 μg/ml [9, 10]. Since the therapeutic to toxic effect ratio is low and the pharmacokinetics of mexiletine are highly dependent on the

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patient's condition, individualization of drug dosage is important [11, 12]. Therefore, routine monitoring of plasma levels of mexiletine will be helpful to obtain effective arrhythmia suppression without toxicity.

For the determination of mexiletine in biological fluids a number of gas chromatographic methods have been described which require flame ionisation detection [13—17], electron-capture detection [18—20], or nitrogen-selective detection [21—24]. However, only three of these methods [18, 19, 24] are sufficiently sensitive to quantitate mexiletine concentrations below 100 ng/ml. To our knowledge, there are no other published assays for the determination of mexiletine. We describe here a sensitive method for the rapid analysis of mexiletine by high-performance liquid chromatography (HPLC) following derivatization with dinitrofluorobenzene. Its applicability for drug monitoring and pharmacokinetic studies in plasma, urine, and cerebrospinal fluid is demonstrated.

EXPERIMENTAL

Materials

Mexiletine [1-(2,6-dimethylphenoxy)-2-aminopropane], the metabolites 4-hydroxymexiletine [1-(2,6-dimethyl-4-hydroxyphenoxy)-2-aminopropane] and 2-hydroxymethylmexiletine [1-(2-hydroxymethyl-6-methylphenoxy)-2-aminopropane], and the internal standard 4-methylmexiletine [1-(2,4,6-trimethylphenoxy)-2-aminopropane] were obtained from Boehringer Ingelheim (Ingelheim, G.F.R.). 2,4-Dinitrofluorobenzene (purity approx. 98%; Sigma, St. Louis, MO, U.S.A.) was used without further purification. Tests for interfering peaks were performed with plasma samples spiked with 10 μg/ml of the following drugs: lidocaine (Xylocain^{R} Astra), bretylium tosylate (Bretylol^{R} American Critical Care), phenytoin (Phenhydan^{R} Desitin), disopyramide (Rythmodul^{R} Albert-Roussel), propafenone (Rytmonorm^{R} Knoll), aprindine (Amidonal^{R} Madaus), chinidine (Chinidin-Duriles^{R} Astra), ajmalin (Giluryt-mal^{R} Giulini-Pharma), verapamil (Isoptin^{R} Knoll), propranolol (Dociton^{R} ICI-Pharma), acebutolol (Prent^{R} Bayer), metoprolol (Beloc^{R} Astra), pindolol (Visken^{R} Sandoz), dihydralazine (Nepresol^{R} Lappe), methyldopa (Presinol^{R} Bayer), dopamine (Cardiosteril^{R} Fresenius), dobutamine (Dobutrex^{R} Lilly), furosemide (Lasix^{R} Hoechst), nitroglycerin (Trinitrosan^{R} Merck), aminophyllin (Euphyllin^{R} Byk Gulden), morphine (Amphiole^{R} Merck), meperidine (Dolantin^{R} Hoechst), pentazocine (Fortral^{R} Winthrop), diazepam (Diazepam-ratiopharm^{R} Ratiopharm), and cimetidine (Tagamet^{R} SK Dauelsberg). All other substances were of analytical-reagent grade and were used without further purification.

Sample preparation

For routine monitoring samples of 0.5—1 ml blood were collected in tubes containing 50 units of heparin (10 μl of Thrombophob^{R} Nordmark, Hamburg, G.F.R.). After centrifugation at 8000 *g* for 5 min (Microfuge 5412; Eppendorf, Hamburg, G.F.R.), plasma samples of 0.2—0.4 ml were transferred to 15-ml capacity PTFE-lined screw-cap liquid scintillation vials (Greiner, Nürtingen, G.F.R.), followed by the addition of 1.2 or 1.0 ml of water, 0.2 ml of internal

standard (2 μg of 4-methylmexiletine), 0.5 ml of 1 *N* sodium hydroxide, and 5 ml of diethyl ether. Each sample was shaken for 1 min and dispersed for 3 min in an ultrasound bath. The organic and aqueous phases were separated by freezing in a dry-ice—acetone bath. The upper organic phase was poured into a second liquid scintillation vial, and evaporated at 60°C. Ether extraction was repeated two times. For derivatization 2.5 ml of 2.5% disodium tetraborate and 0.15 ml of 4% (w/v) dinitrofluorobenzene in dioxane were added to the vials, which were then sealed, shaken for 15 sec and held at 60°C for 20 min. The samples were found to be stable at room temperature for at least 1 h. Within this time 2 ml of each sample were injected directly into the chromatograph without further pre-clean-up procedures.

For pharmacokinetic studies at mexiletine concentrations expected to be below 50 ng/ml, ether extraction was performed with plasma volumes greater than 2 ml.

Urine samples (undiluted) and cerebrospinal fluid were treated in the same way. All native samples and evaporated ether extracts that were not analyzed immediately, were sealed and stored frozen at −16°C.

Chromatography

For enrichment of dinitrobenzene-mexiletine and dinitrobenzene-methyl-mexiletine an alternating pre-column switching technique was used similar to a method reported recently [25]. By the use of a column switching module Gynkotek SE-2 (Gynkotek, Munich, G.F.R.) two pre-columns were alternatingly connected to a Model 7125 sample injection valve (Rheodyne, Berkeley, CA, U.S.A.) equipped with a 2-ml sample loop, and an HPLC pump (Model FR-4S,

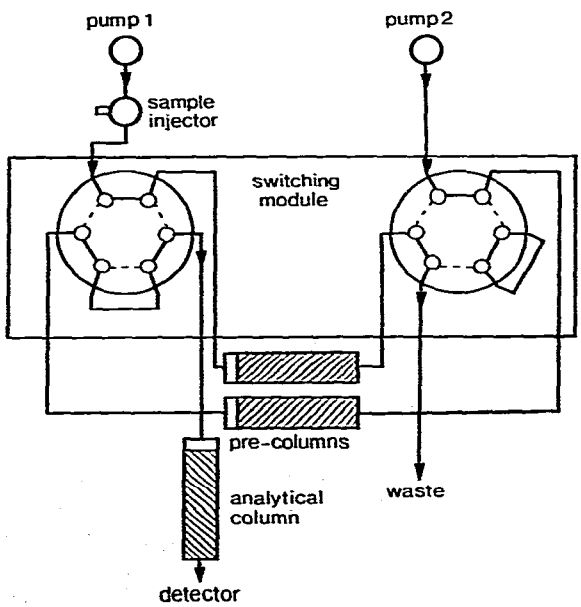

Fig. 1. Scheme of alternating pre-column switching technique for sample enrichment on the pre-column(s) and backflush elution to the analytical column. Further details are described in the text.

Spectra-Physics, Darmstadt, G.F.R.). As illustrated in Fig. 1, sample enrichment on the first pre-column and backflush elution from the second pre-column to the analytical column occur simultaneously. For backflush elution and chromatography on the analytical column a second HPLC pump (Model 600/200, Gynkotek) and a Uvikon 720 LC variable-wavelength ultraviolet detector (Kontron, Eching, G.F.R.) were used.

The pre-columns (5 cm × 4 mm I.D.) were dry-packed with Perisorb^R RP-2 30—40 μm (Merck, Darmstadt, G.F.R.). The analytical column (30 cm × 4 mm I.D.) was packed by forcing a slurry of 4 g of Spherisorb^R ODS 5 μm suspended in 40 ml of carbon tetrachloride into the stainless-steel tube under a pressure of 400 bars by means of a type MS 80/8 high-pressure membrane pump (Orlita, Giessen, G.F.R.). The pre-columns were routinely discarded after 200 injections of 2-ml samples.

The sample enrichment on the pre-column was operated by pump 1 (Fig. 1) with water as mobile phase at a flow-rate of 2 ml/min and a pressure of 20 bars at room temperature. The water-soluble co-products of the samples were eluted for 6 min into the waste. Then the pre-column with the enriched sample was switched to the eluent stream of pump 2, and the sample was directly administered onto the analytical column by the backflush mode. Parallel to this process, the other pre-column was switched to the water stream of pump 1, and 3 min later the next 2-ml sample was injected into the chromatographic system. For chromatography on the analytical column a ternary system of 1-heptanesulfonic acid (0.005 *M*; PIC B7, Waters, Königstein/Ts, G.F.R.)—acetonitrile—tetrahydrofuran (42:48:10, v/v) was used as eluent. The flow-rate was 2.4 ml/min at a back pressure of 230 bars at room temperature. Detection was set at 0.015 a.u.f.s. and at 352 nm, the absorption maximum of dinitrobenzene-mexiletine in the eluent.

For quantitation the areas under the curves were computed by an SP4100 computing integrator (Spectra-Physics). Calibration was performed by the method of internal standardization.

RESULTS AND DISCUSSION

Ether extraction from plasma at a concentration of 0.2—5 μg yielded a cumulative recovery of 46—69% for mexiletine, and of 49—74% for 4-methylmexiletine.

Since the molar extinction coefficient of mexiletine at λ_{max} (260 nm) was only 255 in 0.01 *N* HCl (d = 1 cm), mexiletine was not detectable in our chromatographic system at concentrations below 50 μg/ml. Therefore, the primary amino groups of mexiletine and 4-methylmexiletine were coupled with the chromophore 2,4-dinitrofluorobenzene, similar to methods described for the analysis of cyclohexylamine in cyclamates [26, 27]. Derivatization of mexiletine and 4-methylmexiletine was optimal and sufficiently reproducible at 60°C using dinitrofluorobenzene in a final concentration of 0.24%. The reaction was completed within 20 min. The dinitrobenzene derivatives were found to be stable for 60 min when stored at room temperature.

Dinitrobenzene-mexiletine and dinitrobenzene-4-methylmexiletine were selectively enriched from 2-ml reaction samples on RP-2 pre-columns using

water for elution of the excess of dinitrofluorobenzene and of other water-soluble co-products. The purge phase with simultaneous sample enrichment on the top of the pre-column was terminated at 6 min by automatic switching of this pre-column to the solvent stream of the second pump, thus delivering the ternary eluent, necessary for separation and chromatography, in the backflush mode from this pre-column to the analytical column. Parallel to this process, the other pre-column is switched to the water eluent of the first pump. After an equilibration phase of 3 min this pre-column was loaded with a further 2-ml sample. Sample injection was done manually, but automatic sample injection is possible.

Optimal resolution of dinitrobenzene-mexiletine and dinitrobenzene-4-methylmexiletine from endogenous constituents of plasma, urine, and cerebrospinal fluid was obtained by ion-pair chromatography with 1-heptanesulfonic acid using acetonitrile and tetrahydrofuran as organic modifier for the reversed-phase mode of partition. The addition of tetrahydrofuran improved solvent selectivity and provided sharp, symmetrical and well-defined peaks of derivatized mexiletine and internal standard. Of the several available reversed-phase materials, Spherisorb ODS, 5 μm, was the best stationary phase with respect to efficiency, permeability and stability. Therefore, chromatography was performed on a Spherisorb ODS column (30 cm × 4 mm I.D.) with 1-heptanesulfonic acid (0.005 *M*; PIC B7, Waters)—acetonitrile—tetrahydrofuran (42:48:10, v/v) as eluent (2.4 ml/min). In this chromatographic system dinitrobenzene-mexiletine was eluted at 7.5 min and the internal standard (1 μg/ml) at 10 min. The dinitrobenzene derivative of the mexiletine metabolite 4-hydroxymexiletine was eluted at 3 min. Its detection was usually disturbed by endogenous constituents of plasma. The dinitrobenzene derivative of the other mexiletine metabolite 2-hydroxymethylmexiletine, however, was eluted later than 20 min, and was not regarded for analysis. Typical chromatograms of dinitrobenzene-mexiletine and internal standard in plasma, urine, and cerebrospinal fluid are shown in Fig. 2. Constituents of plasma (Fig. 2A—C), urine (Fig. 2D and E) or cerebrospinal fluid (Fig. 2F and G) did not interfere with the resolution of either compound.

Separation was not disturbed by 25 other drugs (see Materials) tested for possible interference. No interfering peaks were found in plasma of patients who were treated with the following drugs: lidocaine, acebutolol, dihydralazine, dopamine, dobutamine, hydrochlorothiazide, triamteren, furosemide, nitroglycerin, isosorbide dinitrate, aminophyllin, pentazocine, diazepam, cimetidine, digoxin, heparin, acetyl salicylic acid, dipyridamole, sulfinpyrazone, allopurinol, and bezafibrate. Twenty-five drug-free blood samples obtained from healthy volunteers provided no evidence that normal components of plasma interfered with the determination of mexiletine and 4-methylmexiletine.

Identification of the mexiletine peak was made by its retention time relative to the retention time of the internal standard. The variation was less than 1%, as demonstrated from day to day with plasma standards.

Quantitation was performed using the method of internal standardization.

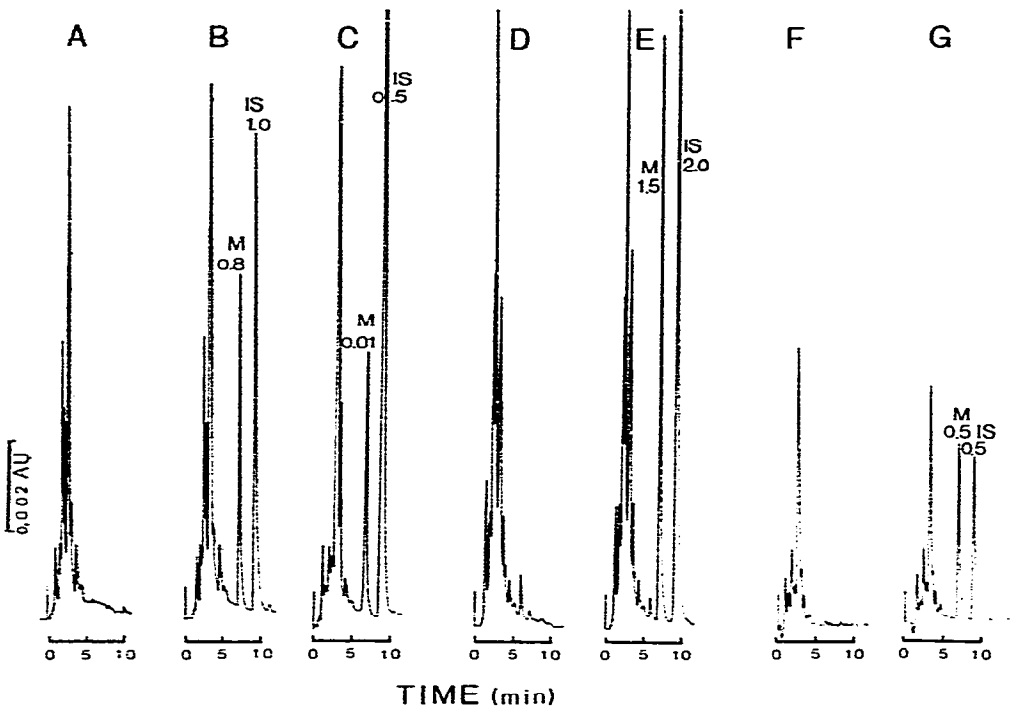

Fig. 2. High-performance liquid chromatograms of mexiletine (M) and the internal standard 4-methylmexiletine (IS) in plasma (B—C), urine (E), and cerebrospinal fluid (G). Traces A, D and F represent the corresponding blanks. The concentrations of mexiletine and of the internal standard are given in μg/ml. The samples were applied onto the Spherisorb ODS 5 μm column (30 cm × 4 mm I.D.) by backflush elution. The eluent, 1-heptanesulfonic acid (0.005 *M*, PIC B7)—acetonitrile—tetrahydrofuran (42:48:10, v/v), was forced at 2.4 ml/min through the analytical column. The back-pressure was 230 bars; room temperature. Detection was at 352 nm.

For mexiletine a linear relationship between peak area and concentration was obtained for the range 0.01—25 μg/ml of plasma. Regression analysis revealed a correlation coefficient that was better than 0.999.

Within-run precision was established in pooled drug-free plasma, which contained 4-methylmexiletine (1 μg/ml) and mexiletine at concentrations of 0.01, 0.02, 0.05, 0.1, 0.2, 1, 5 and 25 μg/ml. For each concentration the within-run precision of six consecutive runs was determined with a coefficient of variation of less than 7%. The day-to-day precision, as determined on six consecutive days for frozen samples of plasma containing 1 μg/ml mexiletine, was found to be 8.6%.

The sensitivity of the proposed HPLC assay allowed quantitation of mexiletine at 50 ng/ml in a 4-ml blood sample and at 10 ng/ml in a 20-ml blood sample with a precision of better than 7%.

The accuracy of the HPLC assay was tested by comparison with a gas chromatographic method ([24]; Boehringer Ingelheim). The correlation between the results obtained by the gas chromatographic method and the HPLC assay is illustrated by Fig. 3. At a *p*-level of 0.05 the mean value of the results obtained by HPLC ($\bar{x}$ = 2.14 μg/ml) was not significantly different from

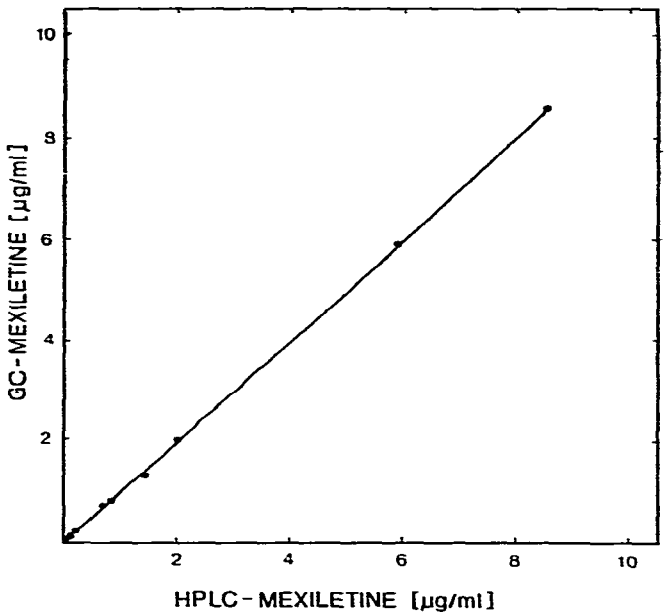

Fig. 3. Mexiletine concentrations in plasma as measured by gas chromatography (GC) and high-performance liquid chromatography (HPLC). Ten plasma samples were spiked with mexiletine in the range 0.05—10 µg/ml. The line was calculated by linear regression analysis.

the value obtained by gas chromatography ($\bar{y}$ = 2.28 µg/ml). The correlation between the results was good (r = 0.998). The slope of the regression line was 1.014, and the intercept was at 0.0068 µg/ml (HPLC values on the abscissa).

The total time for a single analysis is 50 min: 20 min for ether extraction, 20 min for derivatization, and 10 min for chromatography (with sample loading and backflush elution). However, for analysis of multiple samples the speed of analysis is considerably shortened: within 1 h up to five samples can be analyzed.

The stability of the chromatographic system is extremely high. In a series of 200 analyses of 2-ml samples of derivatized mexiletine and internal standard derived from plasma, there was a 30% decline of the efficiency from 28,000 to 19,600 theoretical plates per metre. Replacement of the pre-columns was followed by restoration of the primary efficiency.

Memory effects from the pre-column and/or the sample injector (with the 2-ml loop) were not observed when therapeutic concentrations of mexiletine (0.5—2.0 µg/ml) were injected. However, injection of blank solutions after a run with high amounts of mexiletine (10 µg/ml) revealed a memory effect of about 0.1%.

The proposed HPLC method with alternating pre-column sample enrichment was used for the determination of mexiletine in the plasma of a patient who had received mexiletine for several days. The concentration—time curve of mexiletine is shown in Fig. 4. Adverse symptoms attributable to the drug occurred at plasma concentrations of 3 µg/ml. Apparent side-effects observed in this patient were nausea, vomiting, and nystagmus. Disappearance from

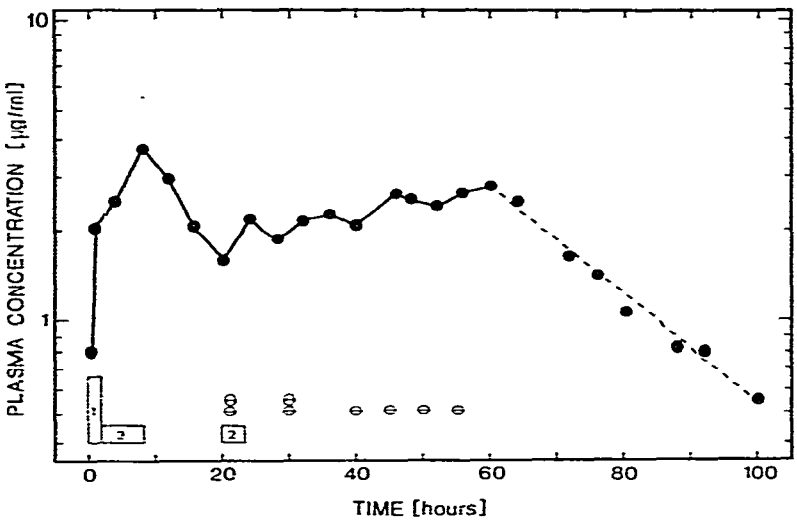

Fig. 4. Time course of mexiletine (•) in a patient's plasma during and following intravenous and oral administration of mexiletine. (1 and 2), infusion of mexiletine with, respectively, 230 mg/h and 60 mg/h (75 kg body weight). (⊖), oral application of mexiletine capsules (Mexitil^{R}, 200 mg). Broken line: monoexponential disappearance of mexiletine as calculated from measured values (r^2 = 0.985). Because of the occurrence of toxic symptoms at plasma levels of about 3 µg/ml, infusion of mexiletine was stoppped for several hours.

plasma was monoexponential with an elimination half-life of 17 h, which is comparable to the half-life values of about 9—16 h reported by others [9, 10].

In summary, a rapid and simple HPLC assay for the analysis of the antiarrhythmic drug mexiletine in plasma, urine, and cerebrospinal fluid is described. Following ether extraction and derivatization to a dinitrobenzene derivative, mexiletine was analyzed using an alternating on-column enrichment technique on small pre-columns in addition to pre-column backflushing for direct injection onto the analytical column. A programmable autosampler can easily be integrated into the chromatographic system thus allowing full automation of the whole analytical procedure. The method is sufficiently sensitive to determine mexiletine at therapeutic concentrations, which are reported to be 0.5—2 µg/ml [9, 10]. The limit of precise determination, 10 ng/ml, allows this HPLC assay to be applied to clinical research and pharmacokinetic studies. Moreover, this method is useful in clinical routine. Rapid monitoring of mexiletine levels in plasma has been shown to be a valuable means of avoiding subtherapeutic or toxic concentrations during antiarrhythmic therapy with this drug.

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