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Determination of MPTP, a toxic impurity of pethidine

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Abstract

Pethidine, predominantly a μ-receptor agonist, is a phenyl-piperidinic synthetic drug. It is used in the management of moderate to several pain. A possible hydrolytic degradation of an ester group can generate a very toxic compound, the *N*-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) which contaminates the drug. Because of the toxicity of MPTP a suitable method for its determination must be selective and sensitive. Afterwards we propose simple methods to determine pethidine and MPTP by capillary electrophoresis (CE), MECK and RP-high performance liquid chromatography (HPLC) looking at the limit of detection obtained using these three techniques. CE was carried out using as running buffer ammonium acetate (pH 8.3). MECK was performed with a borate buffer (pH 8.3) containing sodium dodecylsulphate and trimethyl-beta-cyclodextrins. RP-HPLC was carried out on a RP18 stationary phase, using as mobile phase a mixture of phosphate buffer (pH 7) containing acetonitrile and 1% of diethylamine.

Keywords: Pethidine; Toxic impurity; Capillary electrophoresis; Micellar electrokinetic chromatography; Liquid chromatography

1. Introduction

Pethidine is a phenyl-piperidinic synthetic drug, used in the management of moderate to several pain. Pethidine, ethyl1-methyl-4-phenyl-piperidin-4-carboxylate, is a predominantly μ-receptor agonist.

The pharmacological effects of pethidine are similar to those of morphine, but generally pethidine produces less constipation and urinary retention.

In the recent years the use of pethidine is diminished because of the toxicity of one of its metabolites. Actually a long-term oral or systemic pethidine administration can give rise to an accumulation of the hepatically formed metabolite, normeperidine. An other impurity can be present in pethidine, the *N*-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP), a synthetic substance deriving from hydrolytic degradation of an ester group.

MPTP is a very toxic compound, implicated as the cause of severe and irreversible Parkinsonian symptoms. This impurity causes the destruction of nigrostriatal dopamine neurons, leading to symptoms that closely resemble to those present in humans idiopatic Parkinson's disease [1,2].

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Despite extensive purification of pethidine, the drug may still contains traces of MPTP. This impurity was found in a concentration range of 0.5–5 ppm [3]. In the past the low levels of impurities cannot be considered because of the relative insensitivity of the current analytical techniques. In the last 30 years a lot of sensitive techniques appeared on the market allowing to detect a substance at very low level.

The determination of pethidine was carried out by gas chromatography (GC) with a flam ionization detection or by a packed GC column coupled to an electron capture detector [4–6]. More recently the pethidine was determined in body fluids [7,8] by GC/surface ionization organic mass spectrometry or Capillary GC [9]. The determination of pethidine and MPTP was reported in a review on the determination of drugs of abuse and toxic compounds [10]. Also RP-HPLC was used in the pethidine determination [11,12]. The chemiluminescence techniques [13] allowed a very sensitive determination of this compound. If the determination of pethidine is made using an internal standard, the compound generally used is a butyl ester, homologous of pethidine [14]. In some of these proposed methods the column benefit from the molecular specificity of mass spectrometry was pointed out using several type of the internal standard [15]. Also a capillary electrophoresis (CE) method was used for the determination of various drugs including pethidine [16].

In this paper, we describe the determination of MPTP and pethidine by the most used separative techniques: HPLC, CE and micellar electrokinetic chromatographic (MEKC). The purpose of this paper was to verify the suitability and sensitivity of the three techniques in the determination of MPTP in pethidine.

2. Experimental

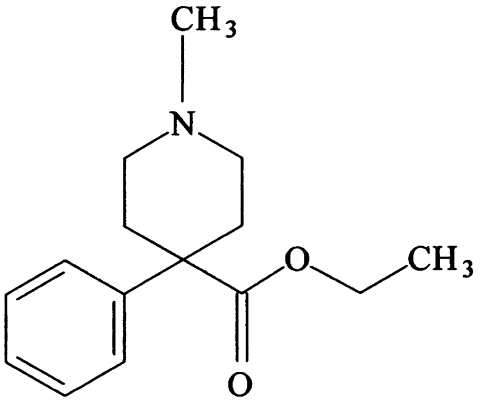
2.1. Chemicals

Pethidine was kindly supplied by the italian 'Istituto Superiore di Sanità' while MPTP (lot 891H4702) was obtained from Sigma (Steinheim, Germany) (Fig. 1). Trimethyl-beta-cyclodextrin were purchased from Sigma-Aldrich (Milan, Italy). Sodium dodecylsulphate (SDS) and all other chemicals were provided from VWR International (Milan, Italy). All chemicals were of analytical or HPLC grade, water included.

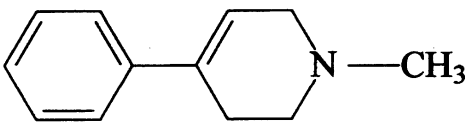
2.2. Instrumentation

2.2.1. High-performance capillary electrophoresis

The CE apparatus was a Cristal CE System (ThermoQuest-Italia, Milano, Italy), equipped with a linear UV–Visible detector (SpectraSYSTEM UV 1000) and an autosampler. The instrument was controlled and the data were evaluated by a Pentium computer. Both CE and MECK analyses were carried out in a 50 μm (I.D.) uncoated fused silica capillary (total length 70 cm, effective length 40 cm Hewlett–Packard CE capillaries, Germany).



PETHIDINE



MPTP = N-methyl-4-phenyl-1,2,3,6-tetrahydro pyridine

Fig. 1. Chemical structures of pethidine and MPTP.

2.2.2. High performance liquid chromatography

The chromatographic separation of MPTP from pethidine was carried out by a Merck-Hitachi Series L-7000 chromatograph, equipped with a Merck-Hitachi Series L-7450 photodiode array detector. The chromatograph was controlled and the data were evaluated by a Flyer Pentium computer, interface D-7000. The detector conditions during the analyses were:

1) acquisition rate of spectra 1600 ms
2) spectral bandwidth for each channel, 4
3) wavelength range: 200–380 nm
4) reference wavelength 450 nm and reference bandwidth 50.

The solutions were injected via a Rheodyne Model 7725 valve using a 20 μl sample loop. The chromatographic analyses were performed using a 5 μm RP-18 column (25 cm × 4.6 mm) obtained from the VWR International (Milan, Italy).

2.3. Analytical procedures

2.3.1. Capillary zone electrophoresis

A 50 mM ammonium acetate (pH 6.5) solution was used as running buffer. The analyses were performed in an uncoated fused-silica capillary, applying a voltage of 20 kV and a working temperature of 25 °C. The samples were injected by applying a pressure of 50 mbar for 3 s. The analytes were detected at a λ value of 200 nm.

2.3.2. Micellar electrokinetic chromatography

The running buffer was a 50 mM borate buffer (pH 8.3), containing 15 mM of SDS and 5 mM trimethyl-beta-cyclodextrins. The analyses were performed by applying a voltage of 20 kV and working temperature of 25 °C. The samples were injected using a pressure of 50 mbar for 3 s. The λ of detection was 200 nm.

2.3.3. High performance liquid chromatography

The HPLC separation of MPTP from pethidine was carried out on a 5 μm RP18 column (Lichrosphere 100, 25 cm × 4.6 mm, obtained from Merck). The column was eluted at 1 ml/min with a mixture of acetonitrile (ACN) and phosphate buffer (55:45) at pH 7. This mobile phase was added with 1% of diethylamine.

2.3.3.1. Standard and working standard analyses. Individual watery standard solutions of pethidine (0.6 mg/ml), MPTP (0.5 mg/ml) and a working standard solutions containing pethidine (0.5 mg/ml) with different amounts of MPTP (1 μg/ml, 0.75 μg/ml, 0.5 μg/ml and 0.25 μg/ml), were separately prepared in a 10 ml volumetric flasks. These solutions were used to study the best analysis conditions for HPLC, CE and MEKC and to verify the linear correlation between absorbance and concentration values. The chromatograms and the electropherograms were recorded at 230 and 200 nm, respectively.

3. Results and discussion

The toxicity of MPTP requires selective and sensitive methods allowing its detection and quantitation. The analysis of MPTP was essentially carried out by GC and HPLC methods. The purpose of this work was to see if the other separative techniques, like CE and MEKC could be equally suitable in the detection and quantitation of pethidine and MPTP. The analyses were carried out by CE, MEKC and also by HPLC using the same working standard solutions, prepared daily. As it was described in Section 2, HPLC analyses were carried out using a RP 18 column as stationary phase. The mobile phase was a mixture of ACN/phosphate buffer (55/45) at pH 7, added with 1% of diethanolamine. The organic modifier was added because it makes easier the exchange between absorption and desorption phenomena of a basic solution. The chromatogram, obtained in these conditions (Fig. 2) showed the good resolution of pethidine ($K' = 2.5$) from its toxic impurity ($K' = 3.4$). Fig. 3 shows the chromatograms concerning to three solutions of pethidine containing 0.25 μg/ml (a), 0.025 μg/ml (b) and 0.0025 μg/ml (c) of MPTP. The minimum of impurity quantitation corresponds to 0.025 μg/ml, while the minimum detectable amount is and 0.0025 μg/ml.

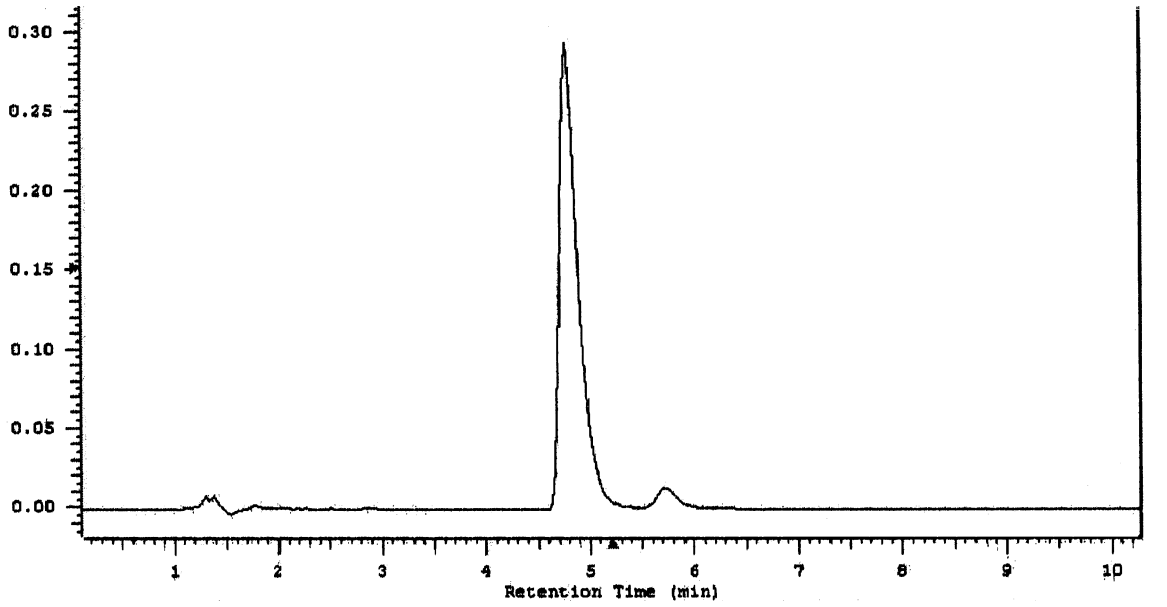


Fig. 2. Chromatogram of pethidine containing 1% of MPTP. Chromatographic conditions: Lichrosphere 100 column, eluted with pH 7 phosphate buffer/ACN (55/45) with 1% of diethylamine at 1 ml/min.

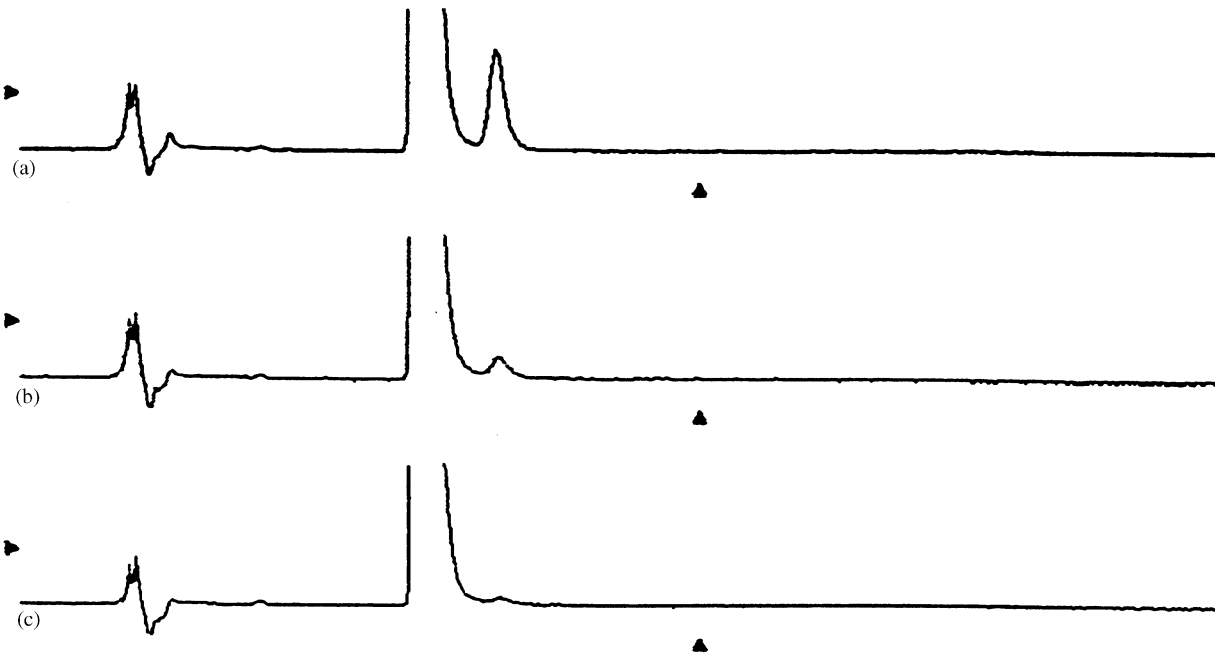


Fig. 3. Chromatograms obtained with pethidine solutions containing (a) 0.25 μg/ml, (b) 0.025 μg/ml, (c) 0.0025 μg/ml of MPTP.

The analyses by CE were performed in an uncoated capillary, filled with 50 mM ammonium acetate buffer (pH 6.5). Fig. 4 shows that pethidine and MPTP are resolved as cations. The sensitivity obtained by CE is entirely similar to that obtained by HPLC. Actually using this technique 0.025 μg/ml of MPTP can be well detected.

By adding to the running buffer (50 mM borate buffer pH 8.3) 15 mM SDS, as micelles generator, and 5 mM of trimethyl-beta-cyclodextrins the best resolution and sensitivity were obtained. As it can see in Fig. 5, the MEKC electropherogram, obtained by injecting a solution containing the same amounts (0.05 mcg/ml) of pethidine and MPTP, shows that the peak of the impurity is higher than pethidine peak. So the sensitivity of the analysis by MEKC seems to be higher than the sensitivity obtained by HPLC and CE. Actually MPTP can be well quantitated also at a concentration of 0.015 μg/ml.

The calibration curves of MPTP and pethidine were performed for each technique using the same standard solutions with at least six different concentrations in the range between 0.0025 and 0.25 μg/ml for MPTP and between 0.25 and 25 μg/ml for pethidine. All the calibration lines showed a good linearity with a correlation coefficients (r) higher than 0.9970 for pethidine and 0.9958 for MPTP. The repeatability of the peak area determination was obtained analyzing pethidine and MPTP solutions (2 μg/ml and 0.01 μg, respectively). The relative standard deviation found was better than 1.8%.

In order to evaluate the accuracy of the methods, we analysed ten samples, obtained from the working standard solution, to which known quantities of MPTP standard were added. The average recovery was about 99.8%.

The precision of the method at each concentration by the intra-assay and the inter-assay was estimated.

4. Conclusions

HPLC, CE and MEKC all allowed a rapid, selective and efficient determination of MPTP in pethidine. The sensitivity of the three separation techniques is similar although the MEKC seems to be a little more sensitive. We can conclude that capillary electrophoretic techniques are complementary to HPLC in qualitative or quantitative

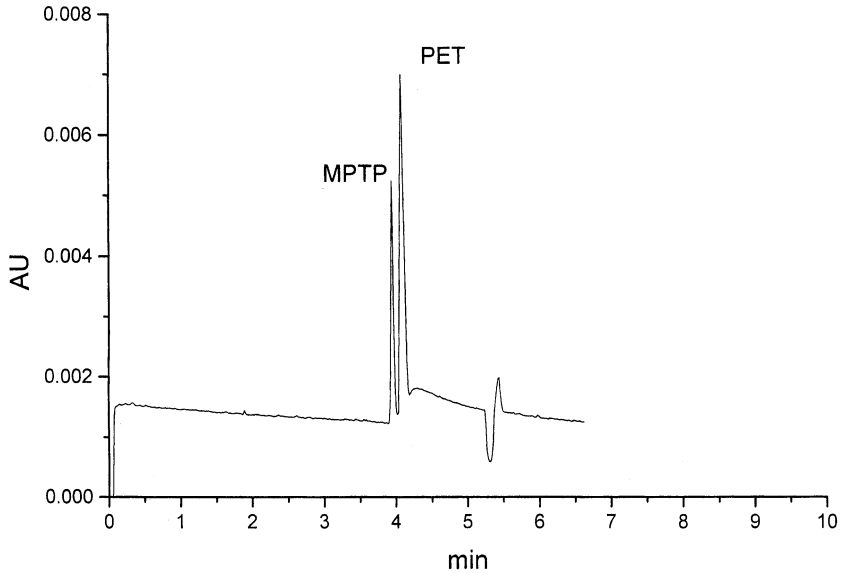


Fig. 4. Electropherogram of a solution containing pethidine (0.15 μg/ml) and MPTP (0.05 μg/ml). Running buffer: 50 mM ammonium acetate (pH 6.5). Applied voltage 20 kV. Temperature: 25 °C. Injection: 50 mbar for 3 s. λ value: 200 nm.

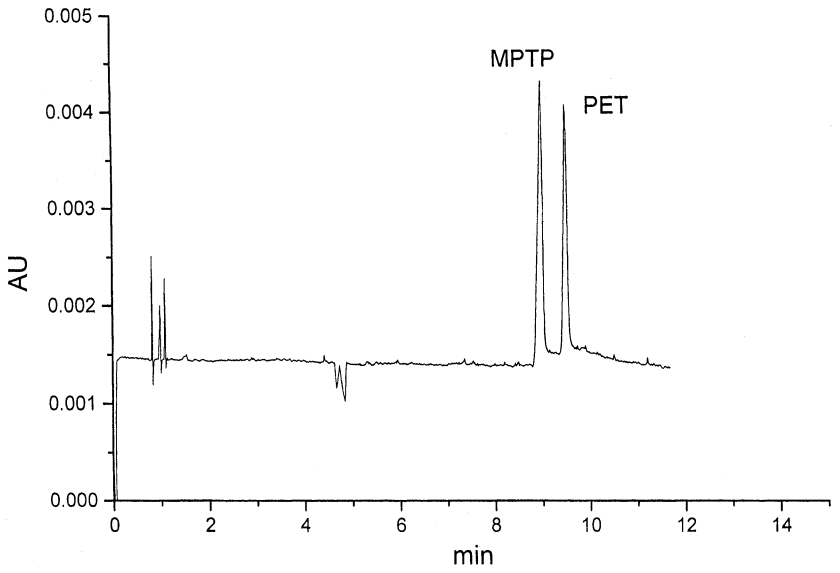


Fig. 5. MEKC analysis: electropherogram of a solution containing pethidine (0.05 μg/ml) and MPTP (0.05 μg/ml). Running buffer: 50 mM borate buffer (pH 8.3) containing 15 mM SDS and 5 mM CD. Applied voltage 20 kV. Temperature: 25 °C. Injection: 50 mbar for 3 s. λ value: 200 nm.

terms. They present the advantage of a low analysis cost, the minimum environmental impact and the high separation efficiency.

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