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Short communication

LC-MS determination of MPTP at sub-ppm level in pethidine hydrochloride

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Abstract

An HPLC-MS with electrospray ionisation method for the determination of MPTP at sub-ppm level in pethidine hydrochloride has been developed and validate. Ionisation is performed by positive-ion electrospray and the quadrupole filter mass spectrometer is operated in the single ion recording mode. Chromatographic separation was achieved in gradient elution using a symmetry C18, 5 μm, 150 mm × 2.1 mm i.d. The mobile phase comprised water containing 0.1% formic acid (v/v) and acetonitrile containing 0.1% formic acid (v/v). The method showed to be linear in the range between 0.2 and 2.2 ng/ml, the estimated LOD was lower than 0.1 ng/ml and the LOQ was lower than 0.2 ng/ml.

Keywords: Pethidine; MPTP determination; Liquid chromatography-mass spectrometry

1. Introduction

The *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a pethidine hydrochloride (PE) impurity (Fig. 1) with a very high toxicity that may have been formed during the process of synthesis. The MPTP was found to be a cause of parkinsonian symptoms due to destruction of nigrostriatal dopamine neurons [1]. The European Pharmacopoeia fixed the following limits for this impurity in the active pharmaceutical ingredient: 10 ppm for PE used for oral preparations and 0.1 ppm for that used for parenteral preparations [2]. The HPLC-UV method described in European Pharmacopoeia [2] is suitable to control MPTP at 10 ppm level whilst it is not applicable to control this impurity at 0.1 ppm. Recently alternative capillary electrophoresis and HPLC methods were developed which proved to be rapid, selective and efficient for the determination of MPTP in PE at level 1–10 ppm [3], but the reported methods are not enough sensitive for the determination of MPTP at 0.1 ppm. Consequently, considering that mass detectors allow to detect substances at ppm or sub-ppm levels present in drugs [4], we decided to use a commercial MS detector coupled with a HPLC apparatus to asses its capability to reach the required sensitivity for MPTP detection at 0.1 ppm. The mass detector used is a single quadrupole based apparatus; it is less sensitive in principle than the more recently developed commercial triple quadrupole MS instrumentations but has the advantage of a lower cost. The development of a simple method for determination of MPTP in PE samples without pre-treatment and which could be applied in routine quality control laboratories by using commercial instrumentation has been our scope.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade acetonitrile and analytical-grade formic acid (98–100%) were obtained from Riedel-de Haën. HPLC-grade water was obtained from BDH (Poole, England). HPLC columns, symmetry C18, 5 μm, 150 mm × 2.1 mm i.d., and XTerra MS, C18, 5 μm, 150 mm × 2.1 mm i.d., were obtained from Waters.

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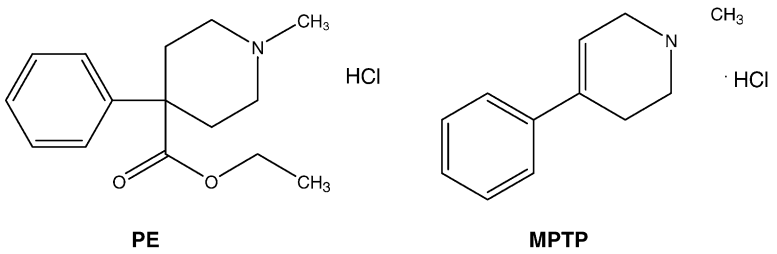

Fig. 1. Chemical structures of pethidine and *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP, purity 100.0%) was obtained from Sigma (St. Louis, USA). Pethidine hydrochloride active pharmaceutical ingredient was supplied from two different suppliers.

2.2. Instrumentation

HPLC-MS analysis was performed using a Waters Alliance 2695 Separation Module coupled to a Waters Micromass ZQ single quadrupole mass spectrometer and a Waters 2487 Dual Absorbance Detector (Waters, Milford, MA, USA). WATERS MILLENNIUM^{32} software (version 4.00) was used for HPLC-MS system control and data collection.

2.3. Method

2.3.1. MS-system

The sample was ionised by electrospray ionisation (ESI) probe in positive ion mode under the following source conditions: source temperature 100 °C, desolvation temperature 350 °C, capillary potential 2.8 kV, sampling cone potential 20 V, extractor 2 V, nitrogen flow rate 500 l/h. Mass chromatograms were obtained in the single ion recording mode (SIR) at 174 *m/z* (MPTP protonated molecule) with low mass resolution value set at 14.0 and high mass resolution value set at 14.0.

2.3.2. Analytical HPLC-system

Chromatographic separation was achieved using gradient elution (Table 1). The mobile phases A and B composition was: water containing 0.1% formic acid (v/v) and acetonitrile containing 0.1% formic acid (v/v), respectively. Degassing of the mobile phase was carried out continuously with an on-line degasser. The UV-detector used for the method development was set at 210 nm. The autosampler temperature was set at 16 °C. The chromatographic run time was 30 min. The injection volume was 10 μl. A three-way valve was fitted in between the column and the mass detector, splitting more than 99% of flow, in order to avoid potential interference of PE (see Section 3.1).

Table 1
Eluent gradient

Time (min)	Flow (ml/min)	Mobile phase A (%v/v)	Mobile phase B (%v/v)
0–1	0.2	99	1
1–6	0.2	99 → 85	1 → 15
6–10	0.2	85	15
10–11	0.2	85 → 40	15 → 60
11–12	0.2	40	60
12–17	0.4	40	60
17–17.5	0.4	40 → 99	60 → 1
17.5–29	0.4	99	1
29–30	0.2	99	1

2.4. Preparation of standard and sample solutions

Standard and sample solutions were prepared dissolving MPTP and PE in HPLC-grade water. PE samples were prepared at 10 mg/ml concentration. MPTP solutions for linearity and accuracy experiments were prepared from 0.014 mg/ml MPTP stock solutions.

2.5. Linearity, accuracy and precision assessment

Linearity was tested for 3 days at six concentration levels of MPTP: 0.1, 0.2, 0.6, 1.0, 1.6, 2.2 ng/ml (corresponding to a range of 0.01–0.2 ppm for 10 mg/ml PE samples). Two independent replicates of each concentration level were injected each day. Linear regression analysis was carried out on the standard curve generated by plotting peak area versus the concentration of MPTP.

Accuracy was tested for 3 days at two concentration levels using 10 mg/ml PE samples spiked with MPTP to obtain a final concentration of 0.8 and 1.2 ng/ml. These concentrations corresponded to 0.08 and 0.12 ppm of impurity. Two independent replicates of each concentration level were injected each day.

These same data were used to assess the precision.

2.6. Limit of detection and quantitation

Limit of detection (LOD) and limit of quantitation (LOQ) were estimated by extrapolation of the calibration curve using the following formulas:

$$\mathrm{LOD} = 3.3\,\sigma/S, \quad \mathrm{LOD} = 10\,\sigma/S$$

where σ is the residual standard deviation of the regression line [5] and S the slope of the regression line.

3. Results and discussion

3.1. Development of the method

Considering that MPTP has an amine moiety, the HPLC-MS experiments were conducted using acidic eluent in order to maximise the formation of the ammonium ion at the mass detector.

The first analyses showed that, even though the mass detector was used in single ion recording mode (SIR) (selecting only the 174 *m/z* corresponding to the $[MPTP + H]^+$ ion) the coelution of PE, in huge amount compared to the MPTP, caused very noisy baseline. Furthermore it was noted the presence of another baseline interference with MPTP appearing only in chromatograms of PE samples, while it was absent in the chromatograms related to the MPTP. This peak is probably due to the high concentration of chloride ions present in PE samples. Therefore the first step of the method development was the optimisation of selectivity in order to obtain a good separation among PE, MPTP and chloride ion. The elimination of any interference was essential, considering the very low level of MPTP concentration to be determined. During method development the UV detector was on line coupled to MS detector. Among the tested columns, using water/acetonitrile/formic acid as mobile phase, the XTerra column and the symmetry column showed the best selectivity. Good separation in isocratic conditions was achieved with the XTerra column when about

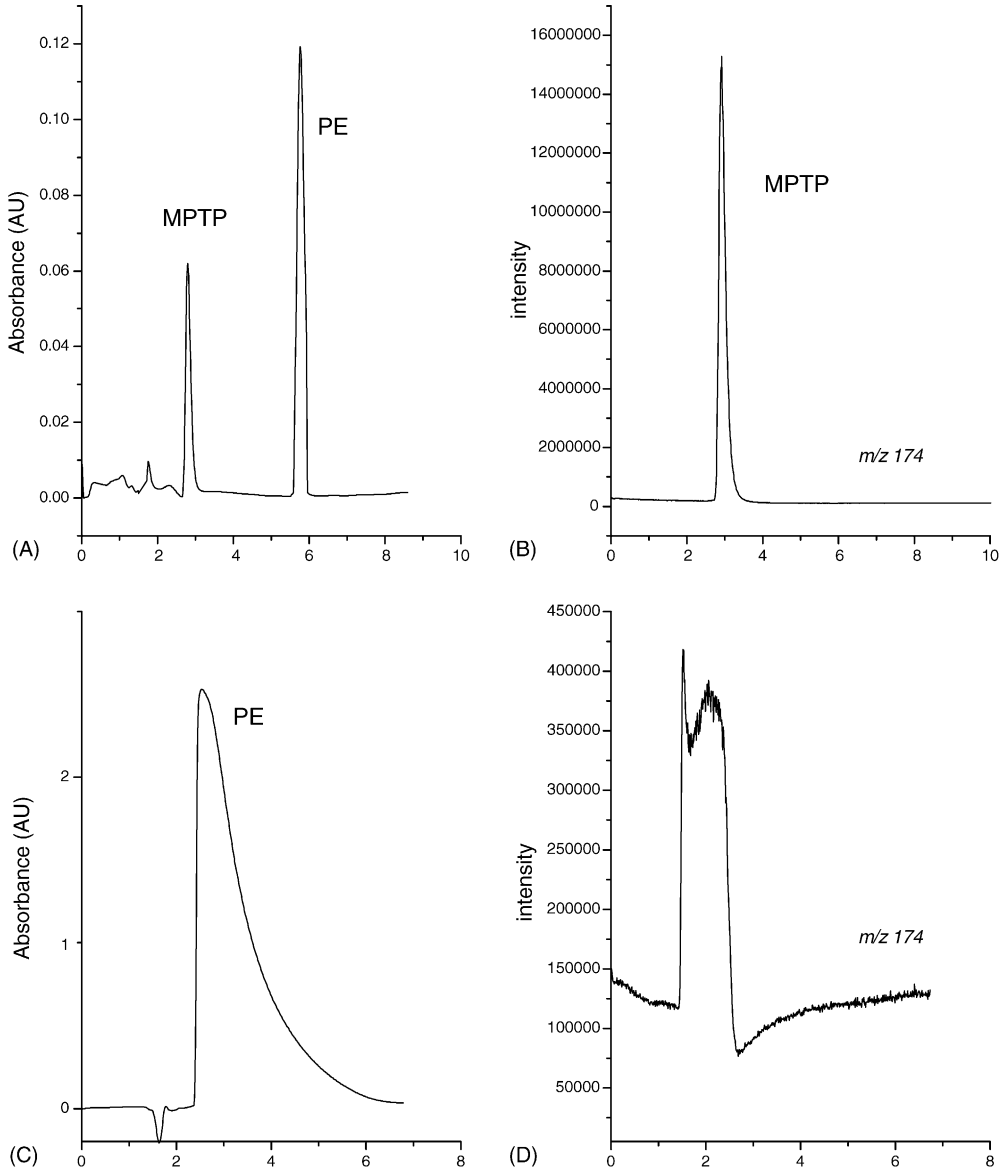

Fig. 2. Analyses performed by the XTerra column. UV (A) and MS (B) chromatograms of about 0.1 mg/ml PE and 0.05 mg/ml MPTP; UV (C) and MS (D) chromatograms of 10 mg/ml PE, injected volume 5 μl.

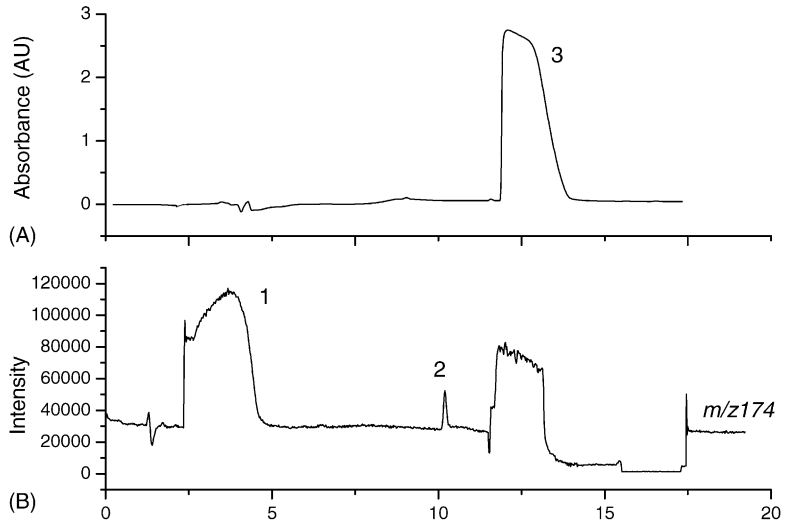

Fig. 3. LC-UV (A) and LC-MS (B) chromatograms using symmetry column. Sample: 10 mg/ml PE containing 1.2 ng/ml MPTP. (1) Chloride ion; (2) MPTP; (3) PE.

1 mg/ml PE spiked with MPTP were injected, but it was not suitable for real samples because, injecting 10 mg/ml PE spiked with MPTP, resolution between MPTP and PE was lost (Fig. 2).

To achieve a suitable selectivity, a gradient elution with the symmetry column was used (Fig. 3); a final washing step was added in order to ensure a thorough elution of PE thus avoiding interferences with the following analysis. During the validation experiments, the MS detector was directly coupled to the column. During the analysis of samples containing PE at high concentration, in order to avoid any potential contamination due to a cumulative effect of repeated injections, the eluent was splitted inserting a three-way valve before the MS detector. In this way, less than 1% of the eluent reached the MS detector. No splitting was operated in the interval between 5 and 12 min of the run (corresponding to elution interval of MPTP).

3.2. Validation of the method

The estimated LOD was lower than 0.1 ng/ml (Fig. 4) and the LOQ was lower than 0.2 ng/ml (corresponding to 0.01

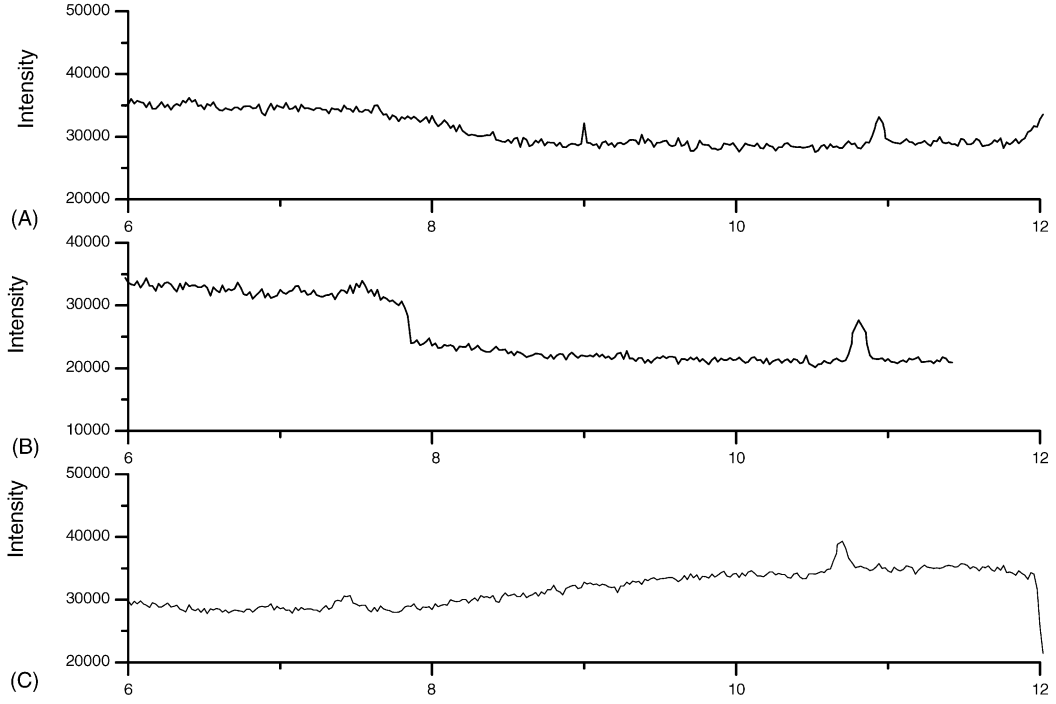

Fig. 4. LC-MS (SIR, *m*/*z* 174) chromatograms using symmetry column: (A) 0.1 ng/ml MPTP; (B) 10 mg/ml PE from supplier 2; (C) 10 mg/ml PE from supplier 1.

Table 2
Calibration curves of MPTP

	Slope	S.D.	Intercept	S.D.	r^2	LOQ (ng/ml)	LOD (ng/ml)
Day 1	169958	1867	1881	2503	0.9996	0.17	0.06
Day 2	197331	2138	−6045	2866	0.9996	0.17	0.06
Day 3	140258	867	9392	1162	0.9999	0.10	0.03

Table 3
Accuracy and precision for 10 mg/ml PE samples

	MPTP added (ng/ml)	MPTP found (ng/ml) ($n = 2$)	Accuracy% ($n = 2$)	Intraday R.S.D.% ($n = 2$)	Interday R.S.D.% ($n = 6$)
Day 1[a]	0.00	0.11			6.0[c]
	0.08	0.90	98.9	6.3	
	1.19	1.29	98.6	5.2	
Day 2[b]	0.00	0.19			
	0.08	0.94	94.9	5.8	
	1.19	1.28	93.0	3.0	4.9[d]
Day 3[b]	0.00	0.21			
	0.08	1.02	101.0	8.0	
	1.19	1.41	100.4	4.4	

[a] PE supplier 1.
[b] PE supplier 2.
[c] Samples spiked with 0.8 ng/ml MPTP.
[d] Samples spiked with 1.2 ng/ml MPTP.

and 0.02 ppm, respectively, for a 10 mg/ml PE sample) as reported in Table 2.

For the assessment of linearity the point at 0.1 ng/ml was not included considering it was lower than LOQ. The method showed to be linear in the range between 0.2 and 2.2 ng/ml (0.02–0.22 ppm) as reported in Table 2.

The accuracy and the intraday and interday precision calculated on MPTP spiked PE samples are listed in Table 3. The accuracy values ranged between 94 and 101% and the values of the precision were in all cases lower than 8%.

4. Conclusions

The data demonstrate that LOD, LOQ, accuracy and precision of the developed LC-MS method for the determination of MPTP in PE samples at sub-ppm levels are adequate to quantify this impurity. Furthermore it has to be noted that the method is simple, considering that there is no need for sample pre-treatment, and it does not require the use of multiple quadrupole MS instrumentation.

The validation data confirm that this method can be used as a limit test for routine analyses and it can be also applied for quantitative determination of impurity.

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