Analytica Chimica Acta 654 (2009) 141–147

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Development and validation of an HPLC method for the determination of process-related impurities in pridinol mesylate, employing experimental designs

Romina M. Bianchini, Patricia M. Castellano, Teodoro S. Kaufman*

Pharmaceutical Analysis, Department of Organic Chemistry, School of Pharmaceutical and Biochemical Sciences, National University of Rosario and Institute of Chemistry of Rosario (IQUIR, CONICET-UNR), Suipacha 531, Rosario (S2002LRK), Argentina

ARTICLE INFO

Article history:
Received 15 July 2009
Received in revised form
15 September 2009
Accepted 16 September 2009
Available online 20 September 2009

Keywords:
Pridinol mesylate
Manufacturing process impurities
HPLC method development
Method validation
Experimental design
Quality control

ABSTRACT

A simple high performance liquid chromatographic method for the determination of process-related impurities in bulk drug of the central anticholinergic compound pridinol mesylate, has been developed and validated. Spectroscopically characterized synthetic impurities were used as standards. The chromatographic separation was optimized employing an experimental design strategy, and was achieved on a C_{18} column with a mobile phase containing 50 mM potassium phosphate buffer (pH 6.4), MeOH and 2-propanol (20:69:11, v/v/v), delivered at a flow rate of 1.0 mL min^{-1}. UV detection was performed at 245 nm. The optimized method was thoroughly validated, demonstrating to be selective, when the chromatogram was recorded with a diode-array detector and peak purities were evaluated (>0.9995). The method is robust and linear ($r^2 > 0.99$) over the range 0.05–2.5% (5–250% with regards to the 1% specification limit for both process-related impurities); it is also precise, regarding repeatability (RSD $\leq$ 1.5% for all of the analytes) and intermediate precision aspects and LOQ values for the impurities are below 0.01%. Method accuracy, evidenced by low bias of the results and analyte recoveries in the range of 99.1–102.7%, was assessed at five analyte concentration levels. The usefulness of the determination was also demonstrated through the analysis of different lots of pridinol mesylate bulk substance. The results indicate that the method is suitable for the quality control of the bulk manufacturing of pridinol mesylate drug substance.

1. Introduction

Process impurities may impact significantly on the final purity and stability of the drug substance, and also complicate its final crystallization step. In addition these impurities, which do not enhance the desired therapeutic effect, may have undesirable adverse effects.

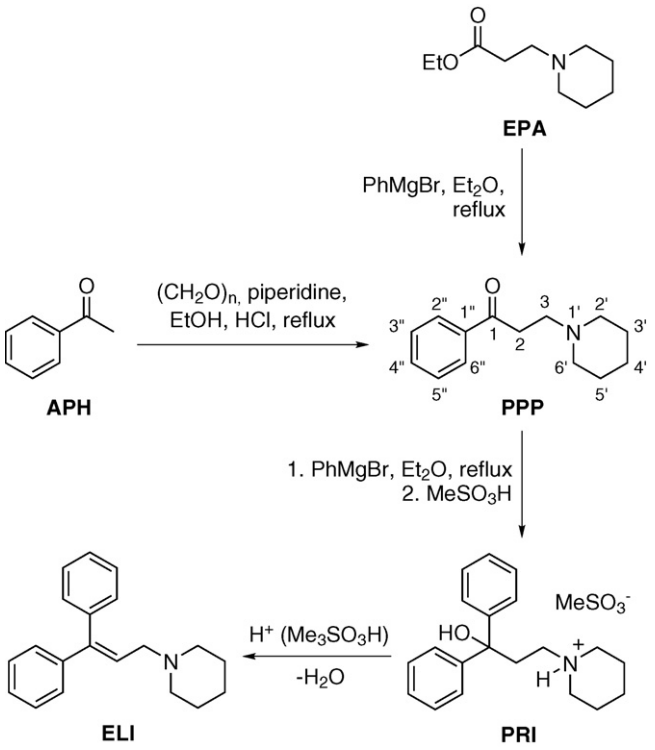
The investigation of process impurities is useful to design control mechanisms for lowering their presence and for setting specifications at appropriate points during manufacture. On the other hand, structural identification of these impurities is important as an approach to hint the chemistry of their formation, being also a key factor in the development of a comprehensive understanding of the commercial manufacturing process [1]. Furthermore, stringent international regulatory requirements for impurities in active pharmaceutical ingredients, as those outlined in the ICH Guideline Q3A, have been approved in recent years [2]. Therefore, process impurities must be controlled, specially in the bulk drugs [3].

Pridinol mesylate (PRI), the methanesulfonate salt of 1-diphenyl-3-piperidinopropan-1-ol (Scheme 1), is a central anticholinergic with useful muscle relaxant properties [4,5] which can be obtained from or through the intermediacy of 3-piperidinopropiophenone hydrochloride (PPP). The drug is used alone in injectable solutions, tablets and patches [6], as a myotonolytic and spasmolytic agent in anti-stress therapy [7] and for the treatment of Parkinson's disease [8,9]. However, PRI is most frequently found in associations with non-steroidal anti-inflammatory agents, including diclofenac, piroxicam and meloxicam [10], which are prescribed for treatment of muscular contractures and low back pain [5,11–13].

We have recently performed stress tests on PRI and developed a stability-indicating assay for the drug [14]; however, the optimized conditions of this assay proved unsuitable for monitoring process impurities of this active principle. In addition, to the best of our knowledge, the chemical structures and the analytical determination of process impurities in PRI bulk drug have not been reported. Therefore, in view of this unfulfilled need, herein we disclose the

* Corresponding author. Tel.: +54 341 4370477; fax: +54 341 4370477.
E-mail address: kaufman@iquir-conicet.gov.ar (T.S. Kaufman).

0003-2670/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2009.09.022



Scheme 1. Chemical synthesis of pridinol mesylate.

identification of process-related impurities of PRI, together with the development and validation of an HPLC method useful for their determination in the bulk substance.

2. Experimental

2.1. Instrumentation

The IR spectrum was obtained using a Shimadzu Prestige 21 FT-IR spectrophotometer (Shimadzu Corp., Kyoto, Japan) with the sample prepared as a KBr pellet; ^{1}H and ^{13}C NMR (proton decoupled) spectra were acquired in $CDCl_3$, employing a Bruker Avance 300 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany); chemical shifts are given in ppm, downfield from tetramethylsilane, used as internal standard and coupling constants (J) are expressed in Hertz. Signals are abbreviated as follows: s = singlet, d = doublet, t = triplet, m = multiplet, b = broad signal, ax = axial and eq = equatorial. The melting point of PPP (uncorrected) was recorded on an Ionomex (Ionomex, Buenos Aires, Argentina) hot stage apparatus.

The HPLC system consisted of a Varian Prostar 210 liquid chromatograph (Varian, Inc., Palo Alto, CA) equipped with two pumps, a manual injector fitted with a 20 μL loop and a Varian Prostar 325 variable dual-wavelength UV–vis detector. The chromatographic separation was performed with a C_{18} column (Luna, 250 mm × 4.6 mm, 5 μm particle size, Phenomenex, Torrance, CA), thermostatized at 30 ± 0.1 °C. The chromatograms were recorded and analyzed employing Varian's Star software.

The selectivity studies were performed by means of a HP 1100 HPLC system, with the above described chromatographic column and employing a diode-array detector. The output signal was monitored and processed using the Chemstation software (Agilent Technologies, Inc., Wilmington, DE). Statistical treatment of the data was performed with SPSS v. 9 (SPSS, Inc., Chicago, IL). Experimental designs were developed and processed employing Design Expert v. 7 (Stat-Ease, Inc., Minneapolis, MN).

2.2. Chemicals and solutions

The HPLC experiments were performed with pharmaceutical-grade PRI (Droguería Saporiti, Buenos Aires, Argentina) and HPLC-grade solvents (J. T. Baker, Phillipsburg, NJ). Chemicals employed for the syntheses of the impurities were acquired from Aldrich Chemical Co., Milwaukee, WI) and used as received. The standard of the impurity PPP was obtained as described below (Section 2.4); the impurity ELI [1-(3,3-diphenylprop-2-en-1-yl)piperidine] was prepared and characterized as previously reported [14]. Stock standard solutions of PRI (10 mg mL^{-1}), ELI (3 mg mL^{-1}) and PPP (3 mg mL^{-1}) were prepared in acetonitrile and stored at 4 °C until use. Solutions for analyses containing mixtures of the analytes were prepared immediately before use, by appropriate dilution of the stock solutions or accurately weighed commercial samples with mobile phase. Phosphate solutions were prepared according to the USP 30 [15], employing double-distilled water. All dilutions were performed in volumetric flasks and the solutions were protected from light throughout the experiments. Liquids were filtered through 0.22 μm nylon filters before use.

2.3. Chromatographic conditions

In the optimized procedure, the mobile phase used for the separation was a 69:11:20 (v/v/v) mixture of MeOH, 2-propanol and potassium phosphate (50 mM, pH 6.4), delivered at a flow rate of 1.0 mL min^{-1}. The organic phase, containing an 85:15 (v/v) mixture of MeOH and 2-propanol, was pumped off from a flask containing the pre-mixed binary solvent. The detection was accomplished at 245 nm.

2.4. Synthesis of the process-related impurity PPP and its spectrometric characterization

Concentrated HCl (1.0 mL) was added dropwise to a solution of piperidine (0.86 g, 0.01 mol), paraformaldehyde (0.45 g, 0.015 mol) and acetophenone (1.2 g, 0.01 mol) in absolute ethanol (3 mL) and the mixture was heated to reflux. After 1 h, an additional amount of paraformaldehyde (0.30 g, 0.01 mol) was added and reflux was continued for another 2 h. Then, boiling acetone (34 mL) was added to the hot mixture and the resulting solution was cooled slowly to room temperature and finally in an ice-water bath. The so produced crystals were collected by filtration, dissolved in hot 95% EtOH (8.6 mL) and the solution was diluted with a fourfold volume of boiling acetone. After cooling to 0 °C (ice-water bath) the resulting crystals were collected by filtration and dried under reduced pressure, yielding 44% of white crystalline material of melting point 192–194 °C. IR (KBr, ν): 2938, 2626, 2549, 1684 (C=O), 1329, 1228, 948, 758 and 696 cm^{-1}; ^{1}H NMR (δ): 1.33–1.47 (m, 1H, H-4′$_{ax}$), 1.66–1.92 (m, 3H, H-4′$_{eq}$, H-3′$_{eq}$ and H-5′$_{eq}$), 2.15–2.30 (m, 2H, H-3′$_{ax}$ and H-5′$_{ax}$), 2.71 (dd, 2H, J = 11.9 and 21.9, H-2′$_{ax}$ and H-6′$_{ax}$), 3.43 (dd, 2H, J = 6.9 and 12.6, H-3), 3.50 (bd, 2H, J = 11.9, H-2′$_{eq}$ and H-6′$_{eq}$), 3.80 (t, 2H, J = 6.9, H-2), 7.44 (t, 2H, J = 7.6, H-3″ and H-5″), 7.57 (t, 1H, J = 7.6, H-4″), 7.97 (d, 2H, J = 7.6, H-2″ and H-6″) and 12.14 (bs, 1H, $w_{1/2}$ = 34, N^{+}H) ppm; ^{13}C NMR (δ): 22.0 (C-4′), 22.6 (C-3′and C-5′), 33.3 (C-2), 52.0 (C-3), 53.8 (C-2′ and C-6′), 128.3 (C-2″ and C-6″), 128.8 (C-3″ and C-5″), 134.0 (C-4″), 135.5 (C-1″) and 196.2 (C-1) ppm.

3. Results and discussion

3.1. Process impurities in PRI. Their origin, chemical synthesis and structural elucidation

The chemical synthesis of pridinol mesylate is outlined in Scheme 1. The drug is commercially obtained by phenyl