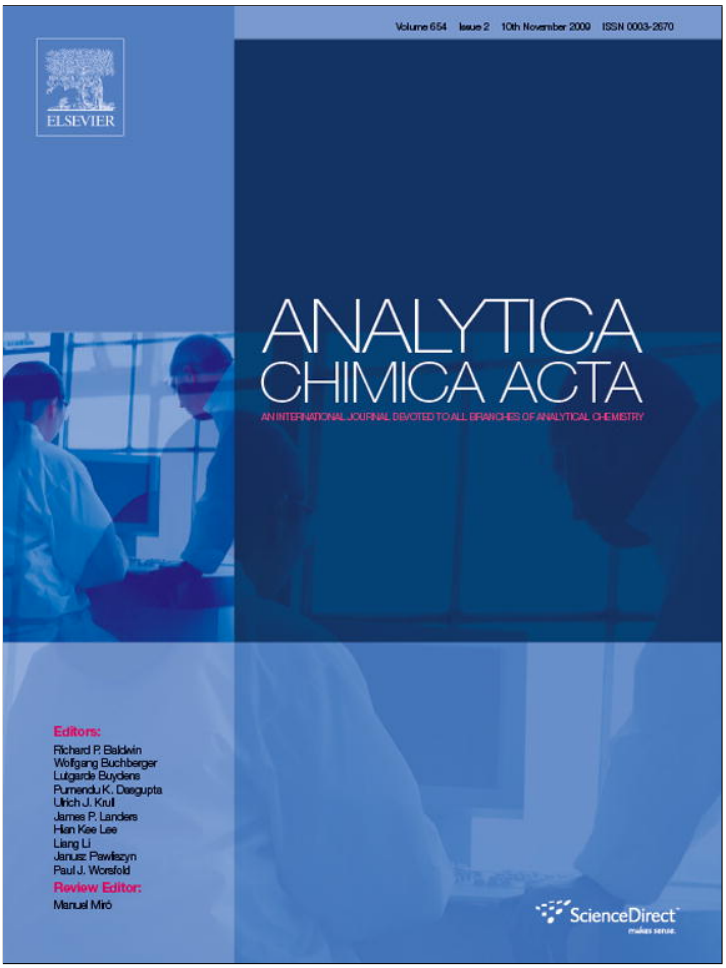

Analytica Chimica Acta 654 (2009) 141–147

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

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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 ≤ 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

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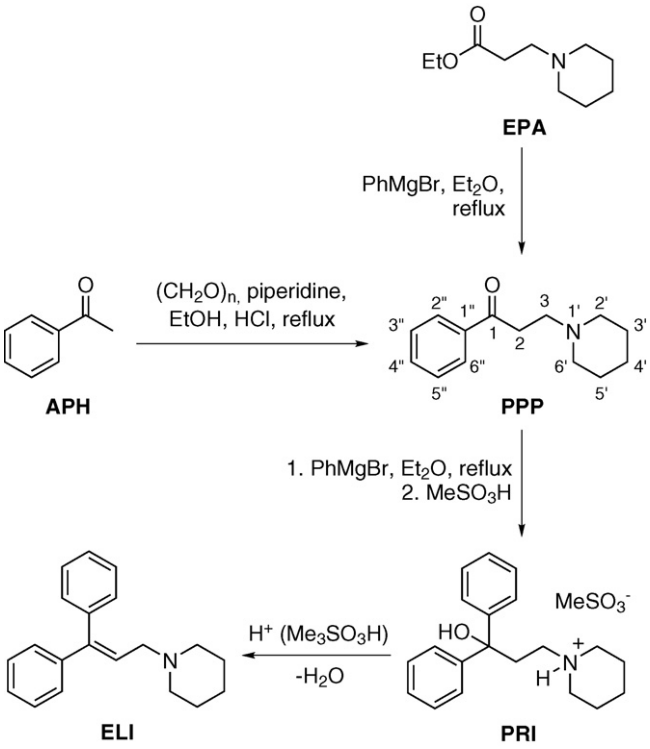
doi:10.1016/j.aca.2009.09.022



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

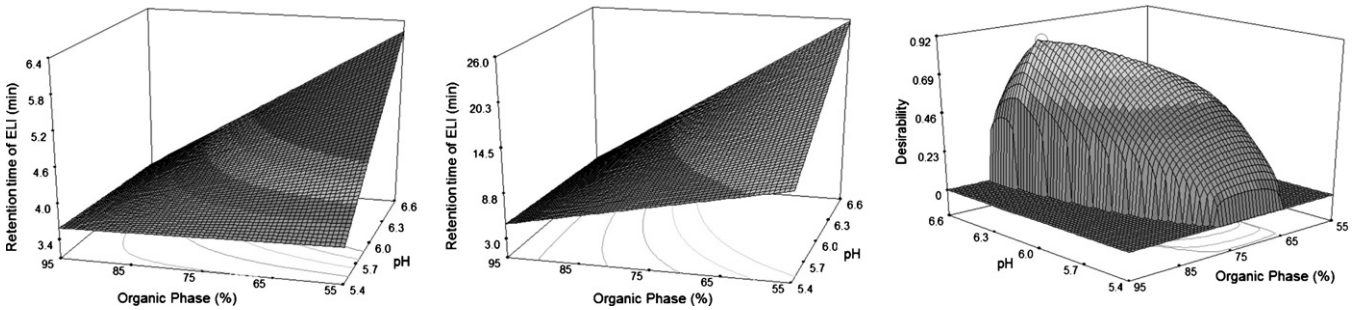


Fig. 1. Effect of the composition of the mobile phase on the retention time of PPP (left), ELI (center) and on the desirability of the separation of PPP, PRI and ELI (right). The white circle indicates the conditions yielding maximum desirability.

Grignard addition (two equivalents) to the ethyl ester of 3-piperidinopropionic acid (EPA), through the intermediacy of PPP. Alternatively, PRI can be prepared by addition of phenylmagnesium bromide to PPP [16,17]; in either case, PPP represents the penultimate intermediate of the synthesis of PRI. Subsequent treatment of the Grignard addition product with methanesulfonic acid yields the mesylate salt. A similar synthetic strategy has been employed for the preparation of analogs of pridinol [18].

Process impurities may result from lack of proper control of the Grignard addition stage, where incomplete reaction could leave unreacted PPP. On the other hand, dehydration of the tertiary and bis-benzylic alcohol resulting in ELI, may take place during the salification step, due to local excess of acid or defective mixing. In systematic stress tests, we have demonstrated that ELI is smoothly produced when PRI is exposed to acidic conditions [14].

The standard of the impurity PPP was prepared by condensation of piperidine, paraformaldehyde and acetophenone (APH) in absolute ethanol under HCl promotion [19]. On the other hand, the standard of ELI was synthesized by forced degradation of PRI with 1N HCl, as previously reported [14]. Both impurities were unequivocally characterized by their melting point and spectral data, including infrared and nuclear magnetic resonance (^{1}H and ^{13}C).

In its FT-IR spectrum, PPP exhibited a characteristic carbonyl absorption band at 1684 cm^{-1}; the presence of the carbonyl moiety was also confirmed by the ^{13}C NMR spectrum of the impurity, which displayed a signal at δ_c = 196.2 ppm. In addition, its ^{1}H NMR spectrum clearly showed three signals corresponding to the *ortho* (δ_H = 7.97 ppm), *meta* (δ_H = 7.57 ppm) and *para* (δ_H = 7.44 ppm) protons of a single phenyl group, and resonances attributable to the piperidine moiety. Resonances at δ 3.43 (dd, 2H, J = 6.9 and 12.6) and 3.80 (t, 2H, J = 6.9) ppm, were assigned to H-3 and H-2, respectively, the two methylene groups connecting the carbonyl to the piperidine ring.

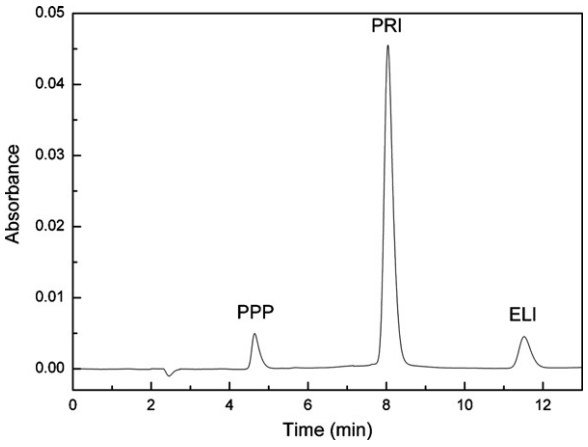


Fig. 2. Typical chromatogram of the separation of PRI (1.0 mg mL^{-1}) from its process impurities PPP (1 × 10^{-2} mg mL^{-1}) and ELI (1 × 10^{-2} mg mL^{-1}).

Interestingly, after performing gram-scale syntheses of PRI, according to the published procedures [16,17], no other impurities were found in the resulting products, confirming that PPP and ELI are the only relevant process-related impurities of this drug.

3.2. Development of an HPLC method for the simultaneous determination of ELI and PPP as relevant process impurities of PRI

With the aid of standards of both impurities, a chromatographic method allowing their separation and quantification was rationally developed, making proper selection of the detection wavelength and the composition of the mobile phase. Based on previous experience and in order to obtain sharper peaks, 2-propanol was added to the MeOH as an organic modifier [14].

3.2.1. Selection of the detection wavelength

In the UV spectra of PRI and its process impurities in the region between 220 and 275 nm, it was observed that ELI has good absorbance below 255 nm, while PPP has a maximum at 245 nm and PRI exhibits comparatively low absorption above 235 nm. Therefore, it was considered that detection at 245 nm would favour sensitivity and precision for the determination of both impurities over detection at lower wavelengths (220–230 nm), where sensitivity of the method is markedly decreased for PPP, preventing its quantification.

3.2.2. Optimization of the composition of the mobile phase

Mixtures of potassium phosphate (50 mM) and (85:15, v/v) MeOH:2-propanol were used for the separation of the analytes. Their composition was optimized with the aid of a 3^2 full factorial experimental design, prepared with nine chromatographic runs under different conditions, which included the pH levels 5.4, 6.0 and 6.6 and the percentage of organic phase levels 55, 75 and 95%. Four responses, including the effects of both factors on the retention time of the first eluting peak, the resolution between each impurity and PRI and the length of the chromatography, at a flow rate of 1.0 mL min^{-1} and employing a C_{18} column, were studied.

It was observed that the retention times of both impurities increased with the increase of the pH of the aqueous phase and also with the decrease of the proportion of the organic solvent in the mobile phase. However, a more in depth analysis of the corresponding response surfaces revealed interactions between these factors (Fig. 1). Therefore, Derringer's desirability function [20] was applied in order to simultaneously optimize the four objective responses.

The combination of factors producing the optimal response, where peak resolutions and the retention time of the first eluting analyte (PPP) were maximized, while the duration of the chromatographic run was kept to a minimum, were 80% of the combined

Table 1
Method precision. Results of the determination of repeatability.

Analyte	Added (mg mL^{-1})	Recovered (mg mL^{-1})	Recovered (%)	RSD (%)
PPP	0.00220	0.00218	99.1	1.2
	0.0110	0.0112	101.7	0.8
	0.0220	0.0226	102.7	0.2
PRI	0.900	0.919	102.1	1.1
	1.000	1.002	100.2	1.5
	1.100	1.108	100.7	0.3
ELI	0.00210	0.00213	101.3	1.0
	0.0100	0.0101	101.0	0.9
	0.0200	0.0203	101.5	0.2

organic solvent and 20% of the 50 mM phosphates solution, at pH 6.4 (Fig. 1). Under these conditions, the overall desirability was 0.88; the closeness of the value to unity indicates the degree of matching of the combined different criteria to the global optimum. Fig. 2 depicts a typical chromatogram, where the retention times of PPP, PRI and ELI were approximately 4.6, 8.1 and 11.5 min, respectively.

3.3. Validation of the HPLC method for the determination of PRI and its process impurities

The optimized HPLC separation was validated for the determination of PRI, ELI and PPP, verifying the aspects established in the corresponding ICH guidelines [21]. Therefore, method selectivity, accuracy, precision (repeatability and intermediate precision), range, linearity and robustness were demonstrated. The limits of detection and quantification for both impurities were determined and system suitability features were also assessed.

3.3.1. Selectivity

The ability of the method to measure the responses of PRI and both of its relevant process impurities without interferences was determined employing a diode-array detector under the optimized chromatographic conditions.

All the analytes were well separated with resolution, $R_s > 2$ between adjacent peaks. In addition, the peak purity function was employed in order to verify the homogeneity of the peaks; values of 0.9997, 0.9998 and 0.9996 for PRI, ELI and PPP, respectively excluded the presence of coeluting interferences embedded in the peaks and confirmed the selectivity of the method. Moreover, forced degradation samples [hydrolytic (neutral, acid and basic), oxidative and photolytic (visible light) conditions, 8-h treatments] produced no additional peaks.

3.3.2. Range and linearity

The linearity of detector response to different concentrations of impurities was studied by analyzing six solutions of PRI, covering the interval 85–115% of the expected concentrations of the analyte. Samples were spiked with both impurities at six concentration levels each, ranging from 0.05 to 2.50%, with regard to PRI (1.0 mg mL^{-1} taken as 100%). The combination of concentrations of the analytes was at random and each solution was injected three times.

The selection of the concentration range of the impurities was effected taking into account current typical tolerance levels (0.1–1.0%) [2] and that impurities below 0.1% do not require quantification unless they are expected to be unusually potent or toxic. The ranges were 0.85–1.15 mg mL^{-1} for PRI and 0.05–2.50% (with regards to PRI at 1.00 mg mL^{-1} taken as 100%) for the impurities.

The data were subjected to statistical analysis using a linear regression model, where they fitted straight lines with correlation coefficients of 0.9942, 0.9999 and 0.9999 for PRI, ELI and PPP, respectively, and the corresponding residuals were distributed at random. These results, which exceeded the requirements set for the test in procedures for the determination of impurities contained in bulk drugs [22], confirmed the linearity of the method for the three analytes within their corresponding ranges.

3.3.3. Precision

The precision of the proposed determination was studied taking into account its repeatability and intermediate precision aspects. In both cases, six independent samples containing three concentration levels of the analytes, distributed at random, were employed; each one was injected three times in random order. The intra-day variation (repeatability) of the assay was expressed as the RSD obtained at the different concentration levels of the analytes. The observed recoveries of PRI and its impurities were almost quantitative (Table 1), and the observed RSD values complied with their typical acceptance criteria (<2.0% for PRI and <10% for ELI and PPP), indicating good method repeatability [12].

For each compound, the intermediate precision was assessed from the inter-day variation recorded on two successive days when the samples were evaluated by three independent analysts. The effects of these variables on drug recoveries were analyzed employing a two-way ANOVA (Table 2); the observed *F*-ratios were smaller than the corresponding critical values revealing that, for the three

Table 2
Method precision. Two-way ANOVA results of the determination of intermediate precision.

Analyte[a] (recovery ± RSD)	Source of variation	Sum of squares	Degrees of freedom	Mean square	*F*-ratio[b]
PPP (99.7 ± 1.5%)	Between analysts	0.46	2	0.23	0.091
	Between days	1.40	1	1.40	0.557
	Days × analysts	1.19	2	0.59	0.236
	Residual	75.47	30	2.52	
	Total	78.52	35		
PRI (100.8 ± 0.9%)	Between analysts	0.68	2	0.34	0.075
	Between days	7.66	1	7.66	1.704
	Days × analysts	1.41	2	0.70	0.156
	Residual	134.95	30	4.50	
	Total	144.70	35		
ELI (101.6 ± 0.9%)	Between analysts	2.18	2	1.09	1.368
	Between days	2.72	1	2.72	3.411
	Days × analysts	2.46	2	1.23	0.230
	Residual	23.92	30	0.80	
	Total	31.28	35		

[a] Six independent samples containing three analyte levels were injected at random, by three different analysts in two different days. Recovery rates and their RSD values are informed for PRI (1.00 mg mL^{-1}) and ELI and PPP (0.01 mg mL^{-1}).
[b] $F_{(0.95,\,1,\,30)} = 4.171$; $F_{(0.95,\,2,\,30)} = 3.316$.

Table 3
Results of the determination of the accuracy of the method.

Analyte	Initial level (%)[a]	Added (%)[a]	Final level (%)[a]	Recovery ($n = 3$) (%, mean ± SD)[a]	Recovery rate (%)	Bias (%)
PPP	0.103	–	0.103	0.105 ± 0.001	101.9	+1.9
	0.103	0.400	0.503	0.504 ± 0.004	100.2	+0.2
	0.103	0.656	0.759	0.761 ± 0.001	100.3	+0.3
	0.103	0.923	1.026	1.021 ± 0.003	99.5	−0.5
	0.103	1.435	1.538	1.532 ± 0.006	99.6	−0.4
PRI	85.0	–	85.0	87.3 ± 0.03	102.7	+2.7
	85.0	5.0	90.0	91.9 ± 0.4	102.1	+2.1
	85.0	15.0	100.0	99.8 ± 0.2	99.8	−0.2
	85.0	20.0	105.0	104.8 ± 0.3	99.8	−0.2
	85.0	30.0	115.0	114.7 ± 0.3	99.7	−0.3
ELI	0.090	–	0.090	0.091 ± 0.002	101.1	+1.1
	0.090	0.360	0.450	0.446 ± 0.006	99.1	−0.9
	0.090	0.576	0.666	0.667 ± 0.004	100.2	+0.2
	0.090	0.810	0.900	0.904 ± 0.003	100.4	+0.4
	0.090	1.242	1.332	1.361 ± 0.004	102.2	+2.2

[a] With regard to a solution containing 1.00 mg mL^{-1} of PRI, considered as 100%.

analytes, the contribution of each studied factor to the total error is significantly smaller than the random error. Therefore, it was concluded there are no significant differences in drug recoveries when the method is applied on different days and by different analysts. The entire evidence confirmed that the method is precise under the proposed conditions.

3.3.4. Accuracy

Accuracy of the method was assessed employing the standard addition method, where samples containing a low level of PRI and its impurities were spiked with the three analytes at four different concentrations; then, the mixtures were analyzed in triplicate. The mean analyte recoveries and their recovery rates obtained for each level, as well as the corresponding bias are detailed in Table 3. Good agreement between actual and determined values and low bias were observed, confirming the accuracy of the determination.

3.3.5. Robustness

The robustness of the method was studied employing an experimental design, where the pH of aqueous phase (6.3–6.5), the proportion of the organic modifier in the mobile phase (78–82%),

Table 4
Results of the determination of method robustness.

Temperature (°C)	Flow rate (mL min^{-1})	pH	Organic phase (%)	Efficiency (N)[a]	Tailing Factor (T_f)[a]	R_s[b]	Recovery (%)[a]
				2,480	1.6		103.7
27.0	0.95	6.3	78	4,890	1.4	8.0, 8.4	101.4
				7,790	1.3		103.3
				3,350	1.6		99.7
27.0	1.00	6.4	80	7,670	1.2	9.5, 8.3	99.0
				8,600	1.3		102.3
				3,450	1.6		97.4
27.0	1.05	6.5	82	6,950	1.4	8.6, 7.0	96.2
				8,040	1.4		98.8
				4,020	1.6		102.2
30.0	0.95	6.4	82	7,070	1.4	8.9, 6.9	101.7
				8,310	1.3		105.4
				3,910	1.7		100.7
30.0	1.00	6.4	80	7,800	1.3	10.2, 8.0	99.0
				8,620	1.3		101.0
				3,670	1.6		100.2
30.0	1.00	6.5	78	8,110	1.2	11.2, 9.2	99.5
				9,600	1.2		102.3
				3,890	1.7		98.0
30.0	1.05	6.3	80	7,530	1.3	9.9, 8.0	96.7
				8,850	1.3		97.6
				4,060	1.6		99.7
33.0	0.95	6.5	80	8,020	1.3	10.5, 8.1	101.6
				9,590	1.2		105.2
				4,270	1.7		98.2
33.0	1.00	6.3	82	7,690	1.3	9.4, 7.0	97.9
				9,110	1.3		102.4
				4,140	1.6		98.2
33.0	1.05	6.4	78	8,660	1.1	11.6, 9.2	94.3
				10,050	1.2		97.5

[a] Top: PPP; middle: PRI; bottom: ELI.
[b] Top: resolution between PPP and PRI. Bottom: resolution between PRI and ELI.

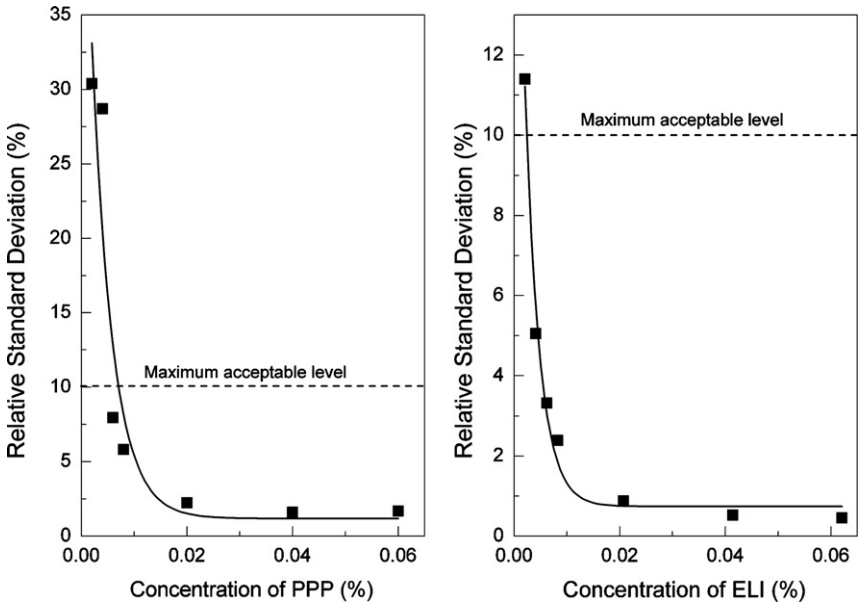


Fig. 3. Determination of the LOQ for the process impurities PPP (left) and ELI (right).

the temperature of the chromatographic separation (27.0–33.0 °C) and the flow rate of the mobile phase (0.95–1.05 mL min^{-1}) were purposely subjected to small modifications. Thirty chromatographic runs were performed in a 3^{4-2} factionary factorial design fashion, and the combined effects the four factors on the characteristics of the chromatogram of PRI and its impurities, including efficiency, analyte recovery, resolution and tailing factor, were evaluated.

The results (Table 4) indicated that the impact of the variations was within acceptable limits. Except under severely adverse conditions, the recovery of the analytes was in the range 100 ± 3%, the tailing factors did not exceed 1.7 within the considered experimental domain, with coefficients of variation below 5% for the impurities and below 10% for PRI; the column efficiencies were always superior to 2000 theoretical plates, being their variation less than 15% in the experimental domain; On the other hand, the resolution values were higher than 7.0, with coefficients of variation below 12%. These data clearly evidenced the robustness of the method.

3.3.6. Limits of detection (LOD) and quantification (LOQ) of the process impurities

The LOQ values were determined for both impurities by the procedure recommended by Huber [23], where RSD values for repeated determinations of the analyte in the neighborhood of the LOQ where plotted against their concentrations. The LOQs were taken as the impurities' concentrations that can be determined with RSD = 10%, although less stringent criteria are also acceptable [24,25]. As shown in Fig. 3, LOQ values for PPP and ELI were found to be 71 and 24 ng mL^{-1} (0.007 and 0.002% relative to PRI, respectively), which are below the currently required reporting threshold for impurities. Considering the limit of detection as 3.3 times lower than the corresponding LOQ, detection limits of PPP and ELI were estimated as 0.002 and 0.0008% relative to PRI.

3.3.7. System suitability test

The test was carried out by performing five replicate injections [15,26] of a solution of PRI (1.0 mg mL^{-1}) containing 0.2% of PPP and 1.0% of ELI. In all cases, resolution between peaks higher than 2.0 and RSD values below 2.0% for the repeated injections were observed (Table 5) and other measured parameters were also within acceptable limits, confirming the system suitability.

3.3.8. Stability of the solutions

Methanolic solutions of PPP proved to be slightly unstable, presumably due to the ketalization of the carbonyl with the solvent; therefore, the solutions of the impurities were prepared in acetonitrile and their stability was assessed in samples stored either 14 days at 4 °C or 48 h at room temperature. In both cases, peak shapes and retention times of the analytes were not affected, no additional peaks were detected, no changes in the chromatographic pattern were observed and samples proved to comply with the following criterion: the relative analyte concentration difference between the beginning and the end of the determination times did not exceed the relative error of determination of the analyte [22]. These were considered as satisfactory evidences of sample stability.

3.4. Application of the HPLC method to samples of PRI bulk substance

The validated method was applied to samples from nine different batches of PRI bulk drug. The retention times of the impurities

Table 5
HPLC system suitability parameters[a].

Analyte	k	RRT	α	R_s	N	T_f	RSD (%)
PPP	0.90	0.58	2.52	10.0	3620	1.5	1.13
PRI	2.27	–	–	–	7870	1.3	0.51
ELI	3.64	1.42	1.60	7.9	8790	1.3	0.32

[a] k: capacity factor [dead volume (t_0) = 2.45 min]; RRT: relative retention time; α: selectivity; R_s: USP resolution; N: number of theoretical plates; T_f: USP tailing factor; RSD: relative standard deviation (five injections).

Table 6
Determination of the process impurities in commercial batches of PRI[a].

Sample	PPP (%)	ELI (%)	Sample	PPP (%)	ELI (%)
Batch I	0.31	0.05	Batch VI	0.08	0.08
Batch II	0.00	0.00	Batch VII	0.04	0.46
Batch III	0.02	0.10	Batch VIII	0.38	0.22
Batch IV	0.40	0.18	Batch IX	0.14	0.62
Batch V	0.83	0.12			

[a] Solutions of PRI at 1.00 mg mL^{-1} were employed; recoveries of the drug were >99.0%.

matched those of PPP and ELI, no additional impurity peaks were observed and impurity levels were less than 1.0%, complying with conventionally accepted requirements (Table 6). These results indicated the usefulness and suitability of the method for the analysis of real samples.

4. Conclusions

A simple and rapid HPLC method useful for the determination of two process-related impurities in pridinol mesylate drug substance was rationally developed. Detection wavelength and mobile phase composition were optimized by examination of the UV absorption spectra of the analytes and with the aid of an experimental design, respectively. The proposed method was thoroughly validated, demonstrating to be selective, precise, linear in the studied concentrations range, accurate and robust in determining PRI and its process impurities, which may be present at trace levels in the bulk drug. LOQ values for the impurities confirmed method's ability to quantify minor amount of these contaminants. Commercial lots of the active principle were analyzed, demonstrating the suitability of the method and its usefulness for everyday quality control purposes.

Acknowledgements

The authors thank the Secretary of Science, Technology and Innovation (SECTeI), the Argentine National Research Council (CONICET) and the Agency for the Promotion of Science and Technology (ANPCyT) for financial support. Thanks are due to Dr. K. Manco (MAR Laboratories, Argentina) for the provision of some samples of pridinol mesylate.

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