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Method development and validation for the simultaneous determination of meloxicam and pridinol mesylate using RP-HPLC and its application in drug formulations

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Abstract

A simple and reliable reversed-phase high-perfomance liquid chromatographic method has been developed and validated for the simultaneous determination of meloxicam and pridinol mesylate in their synthetic mixtures and combined tablet formulations. Both drugs were separated on a 250 mm × 4.6 mm C_{18} column packed with 5 μm particles. The mobile phase, optimized through an experimental design, was a 51:9:40 (v/v/v) mixture of methanol, isopropanol and 50 mM potassium phosphate buffer (pH 5.9), pumped at a flow rate of 1.0 ml min^{-1}. UV detection was performed at 225 nm. The method was validated in the sample concentration ranges of 33.7–61.8 mg l^{-1} for meloxicam and 8.8–16.8 mg l^{-1} for pridinol mesylate, where it demonstrated good linearity with $r = 0.9989$ and 0.9987 ($n = 15$), respectively. The assay was shown to be repeatable at concentration levels of 70%, 100% and 130%, with relative standard deviation values of 1.09% and 0.82% for meloxicam and pridinol, respectively. For independent 100% level samples, the intra-day precision was 0.4% and 1.0% while the intermediate precision was 0.7% and 1.0% for the drugs. The method demonstrated to be robust, resisting to small deliberate changes in pH, flow rate and composition (organic:aqueous ratio) of the mobile phase. The LOD values were 0.22 and 0.20 mg l^{-1}, while the LOQ were 1.7 and 1.1 mg l^{-1}, for meloxicam and pridinol, respectively. The applicability of the method was demonstrated by determining the drug content of two commercial pharmaceutical formulations, where it exhibited good performance.

Keywords: Meloxicam; Pridinol mesylate; Simultaneous determination; Validation; HPLC

1. Introduction

The determination of low concentration and poorly absorbing analytes in pharmaceutical associations constitutes a challenging problem in current pharmaceutical analysis. Tablets containing the pharmaceutical association between meloxicam and pridinol (15 and 4 mg, respectively) are employed for anti-inflammatory, analgesic and myorelaxing purposes. In this combination, the non-steroidal anti-inflammatory drug and COX-II inhibitor meloxicam [MEL, 4-hydroxy-2-methyl-*N*(5-methyl-2-thiazolyl)-2-*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide] is used to relieve symptoms of pain and inflammation [1,2], while pridinol mesylate (PRI, 1,1-diphenyl-3-piperidinopropan-1-ol methanesulfonate), being a central anticholinergic, acts as muscle relaxant [3]. Both drugs have low solubility in water [4] and their chemical structures are shown in Fig. 1.

The determination of MEL in bulk drug and pharmaceutical formulations has been the subject of intense analytical research, leading to colorimetric [5,6], normal [7–10] and derivative spectrophotometric [11], fluorometric [12,13], polarographic [14,15], voltammetric [16] and electrochemical [17,18] methodologies, as well as procedures based on non-aqueous titration [19], HPLC [20–25], flow-injection-spectrophotometry [8,26], TLC-densitometry [27] and capillary electrophoresis [28,29].

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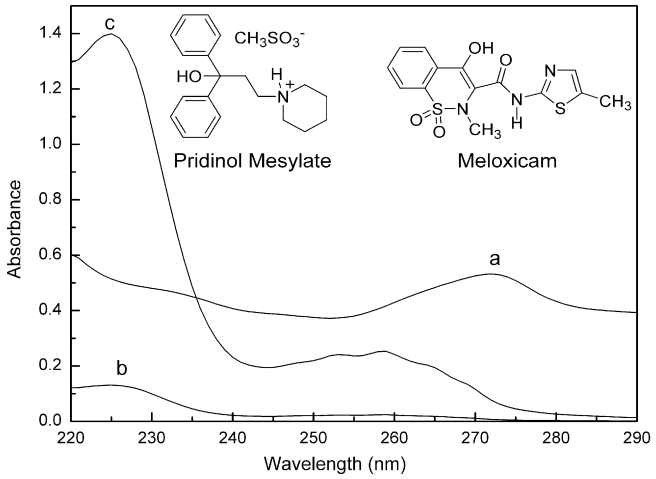

Fig. 1. Chemical structures and UV spectra of: (a) meloxicam (7 mg l^{-1}); (b) pridinol mesylate (1.87 mg l^{-1}) in a 51:9:40 (v/v/v) mixture of methanol, isopropanol and 50 mM potassium phosphate buffer (pH 5.9); (c) pridinol mesylate (20 mg l^{-1}) in the same solvent for optimum wavelength selection.

MEL has also been determined in biological fluids employing HPLC [21–23,30] and LC–MS [31] methodologies. On the other hand, reported methods for the quantification of PRI are very scarce, and include the recent use of GC–MS [32] and capillary electrophoresis for its analysis in biological fluids [33].

A comprehensive literature search revealed the lack of a suitable procedure for the simultaneous determination of these two drugs in pharmaceutical dosage forms. Therefore, the aim of the present work is the development and validation of a simple and reliable HPLC method for the simultaneous determination of MEL and PRI in their combined tablet formulations, and its application to the determination of both analytes in commercial brands of their combined tablet formulation.

2. Experimental

2.1. Chemicals and reagents

All experiments were performed with pharmaceutical-grade MEL and PRI, and analytical-grade reagents. HPLC-grade solvents were employed for analyses. Buffer solutions were prepared with double distilled water according to the USP 30 [34]. Solvents were filtered through 0.47 μm nylon filters. All dilutions were performed in standard volumetric flasks. The pharmaceutical preparations, declaring to contain 15 mg MEL, 4 mg PRI and excipients, were obtained from a local drugstore.

2.2. Instrumentation and chromatographic conditions

The separations were performed with a Varian Prostar 210 liquid chromatograph consisting of two pumps, a manual injector fitted with a 20 μl loop and a Varian Prostar 325 variable dual-wavelength UV–vis detector set at a working wavelength of 225 nm and at an auxiliary wavelength of 259 nm. Compounds were separated on a 250 mm × 4.6 mm C_{18} column (Luna, Phenomenex, 5 μm particle size). The mobile phase was a 51:9:40 (v/v/v) mixture of methanol, isopropanol and 50 mM potassium phosphate buffer (pH 5.9), pumped at a flow rate of 1.0 ml min^{-1}. The organic phase, containing an 85:15 (v/v) methanol–isopropanol mixture, was pumped off from a flask containing the pre-mixed solvent. Chromatograms were recorded employing Varian Galaxie v. 6.0 software. Statistic analyses were performed with Origin v. 7.5 (OriginLab, Co. Northampton, MA, USA).

2.3. Preparation of stock and working standard solutions

The stock solution of MEL (702 mg l^{-1}) was prepared in a 50 ml volumetric flask by dissolving an accurately weighed amount (35.1 mg) of MEL in a mixture of 20 ml methanol and 5 ml 0.1N sodium hydroxide; the solution was completed to the mark with methanol. The stock solution of PRI (400 mg l^{-1}) was prepared in a 50 ml volumetric flask by dissolving in methanol 20.0 mg of accurately weighed PRI. The solutions, which proved to be stable for a period of 3 months, were conserved at 4 °C, in light-resistant containers and were left to attain room temperature before use.

Working solutions were prepared immediately before use, by 1:5 and 1:10 dilutions of the corresponding stock solutions of MEL and PRI, respectively, with mobile phase. Solutions containing mixtures of MEL and PRI were prepared by dilution of appropriate volumes of the working solutions in the mobile phase. All the solutions were protected from light throughout the experiments.

2.4. Sample preparation

Pharmaceutical formulations of two different brands (average weights of 218 and 182 mg/tablet) were evaluated. In each case, 20 tablets were accurately weighed and their average weight was calculated. The tablets were crushed in a mortar to a homogeneous powder and a quantity equivalent to one tablet was weighed and transferred to a 10 ml volumetric flask using a mixture of 4 ml of MeOH and 1 ml of 0.1N sodium hydroxide. The flask was mechanically shaken for 10 min and completed to the mark with methanol. After centrifugation (10 min at 3000 rpm) in order to separate undissolved excipients, 2.5 ml of the supernatant was transferred to a 25 ml volumetric flask and diluted to the mark with methanol. Finally, a 2.5 ml aliquot of this solution was transferred to a 10 ml volumetric flask and diluted to the mark with mobile phase. The process was repeated with five aliquots of tablet powder for each commercial brand. The solutions were filtered through a 0.45 μm nylon membrane filter before the analysis.

3. Results and discussion

3.1. Screening and optimization

3.1.1. Selection of the detection wavelength

The UV spectra of MEL and PRI in a 51:9:40 (v/v/v) mixture of methanol, isopropanol and 50 mM potassium phosphate buffer (pH 5.9), in the region between 220 and 290 nm, are shown in Fig. 1.

In their pharmaceutical association, PRI is nominally four times less concentrated than MEL, the latter having also better absorbing characteristics in the UV region. As observed, MEL exhibits fairly constant absorption throughout the spectrum with a maximum at 274 nm, while PRI shows a maximum at 225 nm. This suggested the latter as the optimum detection wavelength in order to favor the quantification of PRI, the less concentrated component of the mixture.

3.1.2. Selection of the mobile phase composition

After a series of screening experiments, it was concluded that phosphate buffers gave better peak shapes than their acetate and citrate counterparts. It was also observed that mixtures of methanol–isopropanol (85:15) and phosphate buffer with more than 35% of aqueous phase produced satisfactory separations, the addition of isopropanol being useful for improving peak shapes. In order to complete the optimization of the composition of the mobile phase, an experimental design was carried out with methanol–isopropanol–phosphate buffer (51:9:40) mixtures.

For that purpose, response surface methodology (RSM) seemed to be the most suitable experimental design strategy. The goal of RSM is to construct mathematical models that predict how changes in controlled variables, like pH and buffer concentration, affect several responses, including elution time and resolution, in a defined experimental domain. Therefore, a set of nine conditions for the aqueous mobile phase [three different pH values (5.5, 6.0 and 6.5) and ionic strengths (35, 50 and 60 mM)] conforming a full-factorial design, was used to determine the optimal separation conditions, in conjunction with the desirability function approach proposed by Derringer and Suich [35]. In this approach, and in order to make possible the combination of results obtained for properties measured on different scales, the observed responses y_i, ($i = 1,2,\ldots m$), are transformed to a dimensionless desirability scale (d_i), defined as a partial desirability function. The scale of this function ranges between $d = 0$ for an undesirable response, and $d = 1$ for the target value of the response. Once the function d_i is defined for each of the m responses of interest, a global objective function (D), representing the overall desirability function, is calculated by determining the geometric mean of the individual desirabilities. Therefore, D is calculated as the mth root of the product of the partial desirabilities and then, values of the design variables that maximize D can be chosen. A value of D close to 1 indicates that the combination of the different criteria is globally optimal, the response values being near the target values. In this study, this method was employed to simultaneously optimize the resolution of the analytes and the duration of the chromatography as a function of the composition (pH and ionic strength) of the aqueous phase.

It was observed that the retention time (t_r) of MEL slowly decreased with an increase of the pH, while a smooth increase of the t_r was evidenced with increments in the ionic strength of the aqueous phase. On the other side, PRI was more sensitive to pH and ionic strength variations; its t_r increased with the pH, while decreased when the ionic strength of the aqueous phase was incremented. Thus, t_r of PRI was the most influential parameter for defining the resolution between the analytes; it also determined the duration of the chromatographic separation.

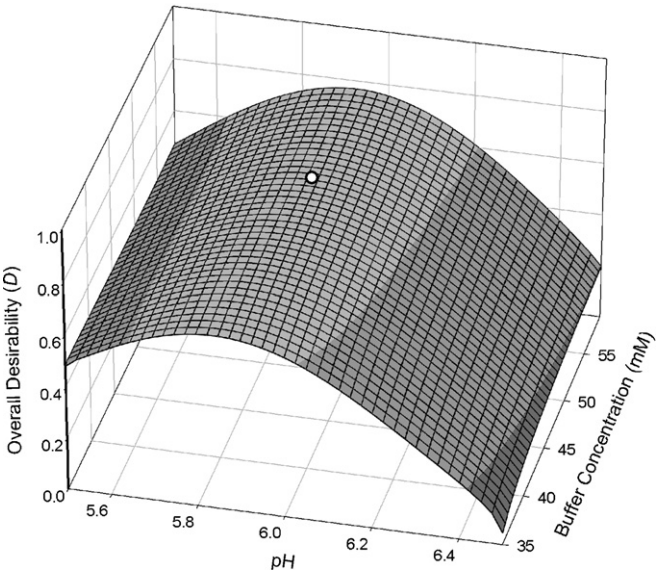

Fig. 2. Response surface of the overall desirability function. The selected working conditions (50 mM phosphate buffer, pH 5.9) are marked as a white dot.

After calculation, it was observed that pH 5.9 represents the optimal pH condition for the separation, with the desirability values increasing with the buffer concentration. However, in order to avoid salt precipitation, as observed in independent solvent mixing experiments, a 50 mM phosphate buffer pH 5.9 was selected for method validation. These conditions gave an overall desirability (elution of MEL, peak resolution and duration of the chromatography) for the studied variables (pH and ionic strength of the aqueous mobile phase) equal to 75%. The overall desirability response surface is shown in Fig. 2. Under these conditions, the retention times of MEL and PRI were 3.66 and 8.30 min, respectively, as shown in the typical chromatogram of Fig. 3.

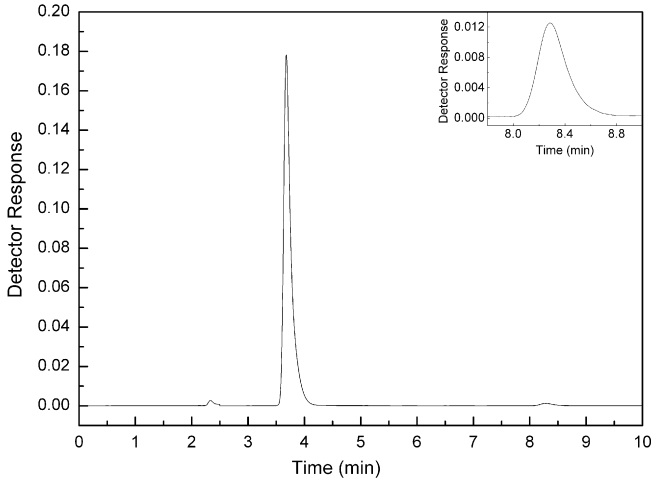

Fig. 3. Typical chromatogram for the separation of MEL and PRI. Top-right: expansion plot of the peak of PRI (10×).

Table 1
Results from the determination of linearity,[a] LOD and LOQ

Analyte	$a \pm$ R.S.D. ($\times 10^5$)	$b \pm$ R.S.D. ($\times 10^8$)	r ($n = 15$)	LOD (mg l^{-1})	LOQ (mg l^{-1})
Meloxicam	-1.8 ± 2.1	3.26 ± 0.04	0.9989	0.22	1.7
Pridinol	-0.5 ± 0.3	1.49 ± 0.02	0.9987	0.20	1.1

[a] AUC = $a + b \times$ drug concentration (mg l^{-1}).

3.2. Method validation

3.2.1. Linearity

Linearity of the proposed method was evaluated according to the ICH guidelines, by the analysis of working solutions of MEL and PRI at five different concentrations [36]. Taking into account the purpose of the assay, the linear ranges were 33.7–61.8 mg l^{-1} for MEL and 8.8–16.8 mg l^{-1} for PRI. These covered the range from 70% to 130% of the expected concentrations of the analytes in the tablet samples. The AUC versus concentration regression data, including the calibration equations and correlation coefficients obtained for both drugs are listed in Table 1. The results show excellent correlations within the tested concentrations ranges.

3.2.2. Limits of detection and quantification

LOD/LOQ parameters are not a requirement for drug assay; however, it is always useful to demonstrate that the analyses are being conducted in a region which is above the LOQ value. The limits of detection (LOD) were established from the standard deviation of the response (S.D.$_a$) and the slope of calibration curves prepared with reference sample solutions having concentrations in the vicinity of the LOD, calculated by the formula LOD = 3.3(S.D.$_a$/b), and assuming that the response–concentration relation is linear in the range from the maximum possible concentration of the analyzed compounds down to zero [37]. The LOD values were 0.22 and 0.20 mg l^{-1} for MEL and PRI, respectively.

The limits of quantification (LOQ) were established according to ICH [36] by the formula LOQ = 10(S.D./b), where S.D. is the standard deviation of the response signal (Table 1); they were 1.7 and 1.1 mg l^{-1} for MEL and PRI, respectively. That the calculated LOQ values allowed confident determination of the analytes was experimentally assessed by injection of samples containing the analytes at their corresponding LOQ concentration values. Under these conditions, PRI and MEL were still accurately determined with satisfactory precision, being 1.2% and 2.6%, respectively, the R.S.D. values of three successive determinations. On the other hand, verification of the proposed LOD values was successfully achieved by visual inspection of chromatograms of solutions of the analytes containing their calculated LOD concentrations.

3.2.3. Precision

Precision was evaluated at the repeatability and intermediate precision levels. Repeatability was studied by the determination of system precision for nine replicate injections of the mixed standard solutions in groups of three, at three different levels [17]. The concentrations studied were 70%, 100% and 130% of the nominal values and the overall relative standard deviations observed were 1.09% for MEL (0.65%, 1.78% and 0.71% for the levels 70%, 100% and 130%, respectively) and 0.82% for PRI (0.94%, 0.85% and 0.35% for the levels 70%, 100% and 130%, respectively).

Intra-assay precision was evaluated by injection of six independent samples at the 100% level [37], furnishing relative standard deviations of 0.4% and 1.0% for MEL and PRI, respectively. Intermediate precision was evaluated by means of a two-way ANOVA of the drug recovery data of six independent mixtures of the standards at the 100% level, injected by three independent analysts, in triplicates, during three different days (Table 2). The overall drug recovery was 100.1 ± 0.7% and 100.2 ± 1.0% for MEL and PRI, respectively. Analogously, for the 70% and 130% levels, the overall drug recoveries were 100.0 ± 0.3% and 100.7 ± 0.5% for MEL and 100.0 ± 0.8% and 100.3 ± 0.4% for PRI, respectively.

Table 2
Intermediate precision: results of a two-way ANOVA for MEL and PRI[a]

Source of variation	Sum of squares	Degrees of freedom	Mean square	F-ratio[b]
Meloxicam				
Between days	1.378	2	0.689	1.317
Between analysts	0.236	2	0.118	0.225
Residual	23.569	49	0.523	
Total	25.183	53		
Pridinol				
Between days	6.068	2	3.034	2.450
Between analysts	0.547	2	0.274	0.221
Residual	55.790	49	1.225	
Total	62.405	53		

[a] Overall mean drug recoveries were 100.1 ± 0.7% and 100.2 ± 1.0% for MEL and PRI, respectively.
[b] $F_{(2,49,0.95)} = 3.1866$.

Table 3
Results from the determination of system suitability

Analyte	Resolution (R)	Tailing factor (T_f)	Theoretical plates (N)	R.S.D. (%) for five separate injections
Meloxicam	14.9	1.8	4700	0.26
Pridinol		1.3	6600	0.70

3.2.4. System suitability

System suitability tests were performed in accordance with USP 30 to confirm that the equipment was adequate for the analysis to be performed. The test was carried out by injecting five replicates of a standard solution containing 47.7 mg l^{-1} and 12.8 mg l^{-1} of MEL and PRI, respectively.

The corresponding observed R.S.D. values were 0.26% and 0.70% which were considered satisfactory, meeting the requirements of USP 30 (R.S.D. <2%). Theoretical plates, resolution and tailing factors were also determined, with their corresponding values listed in Table 3.

3.2.5. Accuracy

The accuracy of the method was determined by measuring the drug recoveries by the standard addition method, in order to determine eventual positive or negative interferences produced by the excipients in the formulation [17].

Known amounts of each drug, corresponding to 90%, 100% and 110% of the label claim were added to a pre-analyzed tablet sample, containing the equivalent to 80% of the label claim of both drugs.

Each set of additions was repeated five times. The results of accuracy, expressed as the percentage of the analytes recovered by the assay; are listed in Table 4. These indicate that the method enables the highly accurate simultaneous determination of both drugs.

3.2.6. Ruggedness

The ruggedness of the proposed method was examined against small, deliberate variations of critical parameters such as the pH, composition of the mobile phase and the flow rate.

The pH was varied in the range 5.8–6.0, the composition of the mobile phase (85:15 methanol–isopropanol:phosphate buffer) was changed from 62:38 to 58:42 (v/v) and the flow rate effect was evaluated between 0.95 and 1.05 ml min^{-1}. The results, shown in Table 5, confirmed the ruggedness of the test, since the observed variations were less than ±1.5%. However, it was observed that the determination of MEL is more sensitive to pH variation than that of PRI and, conversely, the quantification of PRI was more prone to changes with modification of the mobile phase composition than MEL.

3.2.7. Selectivity

Selectivity of the method was demonstrated after observing that the excipients did not produce absorption peaks in the chromatogram and did not interfere with the exact determination of the analytes in the accuracy assay (Section 3.2.5); in addition, chromatograms were completely superimposable with those recorded by simultaneous detection at 259 nm, all of which

Table 4
Results from the determination of the accuracy of the method

Analyte	Concentration (mg l^{-1})				R.S.D. (%)	Recovery (%)	Bias (%)
	Initial	Added	Total	Found	$n=5$		
Meloxicam	12	0	12.0	11.999	0.26	99.99	0.01
	12	1.5	13.5	13.41	0.40	99.37	0.63
	12	3.0	15.0	15.05	0.17	100.36	−0.36
	12	4.5	16.5	16.61	0.44	100.69	−0.69
Pridinol	3.2	0	3.2	3.199	0.78	99.99	0.01
	3.2	0.4	3.6	3.58	0.43	99.42	0.58
	3.2	0.8	4.0	3.98	0.51	99.58	0.42
	3.2	1.2	4.4	4.41	0.37	100.27	−0.27

Table 5
Results from the determination of the ruggedness of the method

Parameter	Meloxicam			Pridinol	
	Value	Mean recovery (%)	R.S.D. (%)	Mean recovery (%)	R.S.D. (%)
pH	5.8				
	5.9	99.5	1.2	99.6	0.5
	6.0				
Flow rate (ml min^{-1})	0.95				
	1.00	100.0	0.2	100.3	0.4
	1.05				
Mobile phase (organic:aqueous)	58:42				
	60:40	100.0	0.1	99.8	0.8
	62:38				

Table 6
Summary of the results of the method validation assays

Parameter[a]	Meloxicam	Pridinol
Linear range (mg l^{-1})	33.7–61.8	8.8–16.8
Accuracy-drug recovery (mean of the bias, %)	−0.41	0.19
Precision		
Repeatability (%)	1.09	0.82
Intra-assay precision (R.S.D., %)	0.4	1.0
Intermediate precision-drug recovery (% ± R.S.D.)	100.1 ± 0.7	100.2 ± 1.0
Selectivity	Excipients do not absorb A_{225}/A_{259} remains constant	
LOD (mg l^{-1})	0.22	0.20
LOQ (mg l^{-1})	1.7	1.1
System suitability test		
R.S.D. of repeated injections (%)	0.26	0.70
Theoretical plates (N)	4700	6600
Tailing factor (T_f)	1.8	1.3
Ruggedness-drug recovery (% ± R.S.D.)		
Variation of pH (±0.1 U)	99.5 ± 1.2	99.6 ± 1.5
Variation of flow rate (±0.05 ml min^{-1})	100.0 ± 0.2	100.3 ± 0.4
Variation of the mobile phase (±2%)	100.0 ± 0.1	99.8 ± 0.8

[a] Chromatographies were carried out with a C_{18} column, employing a 51:9:40 (v/v/v) mixture of methanol, isopropanol and 50 mM potassium phosphate buffer (pH 5.9), pumped at a flow rate of 1.0 ml min^{-1}. The detection wavelength was 225 nm.

Table 7
Assay of meloxicam and pridinol in their combined tablet formulations

Sample No.	Brand No. 1		Brand No. 2	
	Meloxicam (%)[a]	Pridinol (%)[a]	Meloxicam (%)[a]	Pridinol (%)[a]
1	91.9	105.9	95.9	100.4
2	91.3	106.4	94.6	100.2
3	92.1	105.8	94.5	101.4
4	93.1	105.4	95.6	100.3
5	93.8	106.2	95.3	100.3
Mean	92.5	105.9	95.2	100.5
R.S.D.	1.1	0.4	0.7	0.5

[a] Percentage of drug recovered, relative to the label claim.

served as indication that the determination was not interfered by drug degradation products.

The results of the validation assays are summarized in Table 6.

3.3. Application. Assay of pharmaceutical tablets

The validated HPLC method was used for the simultaneous determination of MEL and PRI in their combined dosage form. Five samples of each brand were weighed separately and analyzed. The results, expressed as percentage drug recovery related to label claim, are informed in Table 7. These indicate that the amounts of each drug in the tablets of both brands are within the USP requirements of 90–110% of the corresponding label claims.

4. Conclusions

A simple and efficient HPLC method has been developed, optimized and validated for the isocratic separation and simultaneous determination of meloxicam and pridinol in their combined dosage form. The method, suitable for routine quality control, has been successfully applied to the determination of both analytes in two commercial brands of tablets containing this pharmacological association.

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