

A TURBIDIMETRIC METHOD FOR THE ASSAY OF MELOXICAM USING MOLYBDOPHOSPHORIC ACID

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Abstract

A turbidimetric method was developed for the assay of meloxicam using molybdophosphoric acid in hydrochloric acid medium. The reaction product showed an absorbance maximum at 342 nm. The optimum reaction conditions were established. The developed method was validated. The method showed a good linearity in the range of 1.0 - 2.6 µg/mL (the correlation coefficient $r = 0.9995$). The detection limit (LD) was 0.29 µg/mL and the quantification limit (LQ) was 0.95 µg/mL. There were determined the precision (RSD = 1.79%) and the accuracy (mean recovery was 100.89% in the 98.45 - 103.22% range).

Rezumat

Pentru determinarea meloxicamului s-a dezvoltat o metodă turbidimetrică, bazată pe reacția meloxicamului cu acidul fosfomolibdenic în mediu de acid clorhidric, cu un maxim de absorbție la 342 nm. Au fost stabilite condițiile practice de lucru. Metoda dezvoltată a fost validată. Metoda prezintă o bună liniaritate pe domeniul de concentrație 1,0 - 2,6 µg/mL (coeficient de corelație $r = 0,9995$). Limita de detecție (LD) este de 0,29 µg/mL, iar limita de cuantificare (LQ) este de 0,95 µg/mL. S-a stabilit precizia (RSD = 1,79%) și exactitatea (regăsire medie 100,89% pe domeniul 98,45-103,22%).

Keywords: Meloxicam, spectrophotometric, validation

Introduction

Meloxicam is a nonsteroidal anti-inflammatory drug (AINS) used as an analgesic, antipyretic and anti-inflammatory.

There are methods used for the assay of meloxicam, such as the spectrophotometric methods presented by Nageswara *et al.* [3].

This paper presents a turbidimetric method for the assay of meloxicam using the molybdophosphoric acid in hydrochloric acid medium, reagent used for the assay of other oxicams. The developed method was validated [1, 2, 4].

Materials and methods

Reagents and apparatus:

- Aqueous solutions of hydrochloric acid: 0.5M - 4.0 M;
- Aqueous solutions of molybdophosphoric acid (PMA): 0.1% - 3.0%;
- Sodium lauryl sulphate solution: 0.1%;
- Dimethyl formamide (DMF).

All reagents were purchased from Merck.

- Stock solution (10 µg/mL): 100 mg meloxicam (100.3% pure reference substance) was dissolved in 25 mL DMF in a 100 mL volumetric flask. This solution was diluted to 100 mL with 2M aqueous solution of hydrochloric acid. This solution was diluted 100 times.
- Working solutions containing from 1.0 to 2.6 µg/mL meloxicam were obtained by diluting the stock solution with 2M aqueous solution of hydrochloric acid;
- Analytical balance (Kern 770);
- UV- Vis Spectrophotometer (Hewlett Packard 8453).

Principle of the method: Meloxicam forms with PMA in hydrochloric acid medium an insoluble compound which can be turbidimetrically determined from the suspension at 342 nm.

In order to establish the optimum wavelength for the detection, 3 mL of the 2 µg/mL working solution were mixed with 1% PMA solution and 1 mL 0.1% sodium lauryl sulphate were added. The UV-Vis absorption spectra was recorded using 1 cm cell, after 20 minutes.

In order to establish the optimum working conditions, two solutions of 1.0 µg/mL and 2.6 µg/mL were used (the minimum and the maximum concentration of the linear range), while the parameters of the method were changed. We established the optimum concentration of the hydrochloric acid solution and of the PMA solution and we determined the stability of the reaction product.

Procedure: 1 mL of 1% molybdophosphoric acid solution was added to 3 mL meloxicam solution. In order to prevent the precipitation, 1 mL of 0.1% lauryl sodium sulphate was added. The absorbance was measured at 342 nm (1 cm cell) versus a blank solution prepared in similar conditions.

Method validation:

Linearity: solutions containing meloxicam in a concentration range of 0.5 – 2.8 µg/mL were used. The obtained data was analysed by linear regression and the calibration curve was obtained [5, 6, 7].

Detection and quantification limits were calculated using the following formulas [5]:

$$LD = 3 \times \text{Standard error} / \text{Slope} \quad LQ = 10 \times \text{Standard error} / \text{Slope}$$

Precision: three solutions of 1.4, 1.6 and 1.8 µg/ml meloxicam were used. Three assays were performed for each concentration. Two sets of assays were performed in different days in order to evaluate the intermediary precision.

Accuracy: in order to establish the accuracy of the method, meloxicam solutions of 1.4, 1.6 and 1.8 µg/mL were analysed. For each concentration, three determinations were performed.

Results and discussion

From the analysis of the absorption spectra, we observed a maximum of absorbance for the reaction product at 342 nm. This value was used for all the determinations.

Meloxicam in 2M hydrochloric acid presented a maximum of absorption at 339 nm. The specific absorption coefficient of meloxicam was $A_{1\text{cm},339\text{nm}}^{1\%} = 722$ and the specific absorption coefficient of reaction product was $A_{1\text{cm},342\text{nm}}^{1\%} = 3330$. The measured detection sensitivity increased (4.6 fold).

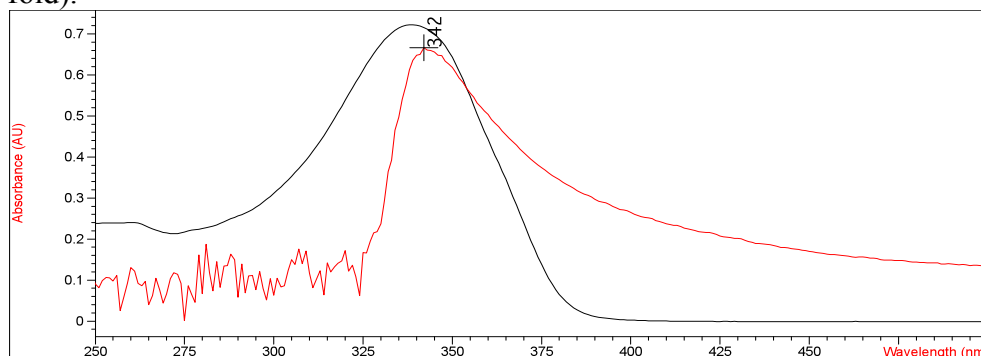


Figure 1

Absorption spectra: (Black: 10 µg/mL meloxicam in 2M hydrochloric acid; Red: reaction product at a concentration of 2 µg/mL meloxicam)

The optimum concentration of the hydrochloric acid solution was set to 2M. The optimum concentration of molybdophosphoric acid was set to 1% (table I). The absorbance was measured after 20 minutes after the last reagent was added (table II). The very low quantity of DMF, remained after

numerous dilutions, didn't interfere with the solubility of the reaction product.

Table I
Reagent concentration

Hydrochloric acid (M)	Meloxicam concentration		PMA conc. (%)	Meloxicam concentration	
	1.0 µg/mL	2.6 µg/mL		1.0 µg/mL	2.6 µg/mL
	Absorbance			Absorbance	
0.5	0.08425	0.029441	0.1	0.03965	0.28510
1.0	0.09522	0.75522	0.5	0.07856	0.68221
2.0	0.10753	0.99145	1.0	0.09952	0.95853
3.0	0.10642	0.99125	2.0	0.10421	0.99963
4.0	0.10477	0.99426	3.0	0.10342	0.99211

Table II
Stability of sample absorbance

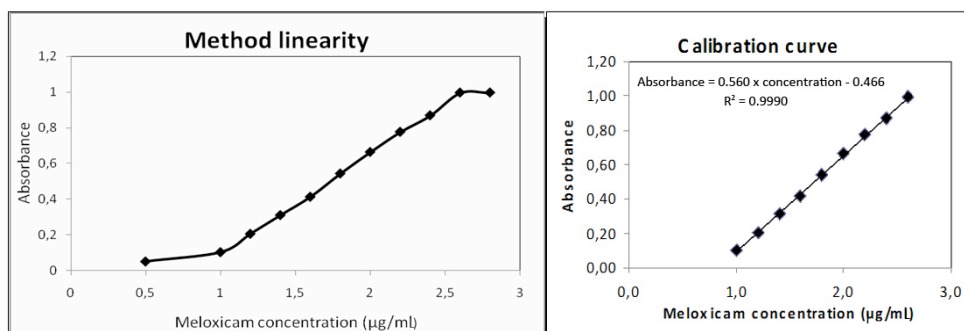
Meloxicam concentration		Time (minutes)					
		10	15	20	25	30	60
1.0µg/ml	Absorbance	0.08752	0.09574	0.09941	0.10441	0.10475	0.10411
2.6µg/ml		0.59562	0.85422	0.95429	0.95429	0.99041	0.99412

Linearity: we studied the linearity in the 0.5 - 2.8 µg/mL concentration range. The obtained data were statistically evaluated (table III) and the calibration curve was obtained (figure 2). In conformity with the experimental data, the developed method for meloxicam determination was linear in 1.0 – 2.6 µg/mL concentration range, the correlation coefficient was $r = 0.9995$. The calibration curve equation was established:

$$Absorbance = 0.560 \times Concentration - 0.466$$

Table III
Method linearity

Meloxicam concentration (µg/mL)	Absorbance				
	I	II	III	IV	Average
0.5	0.04122	0.05847	0.05419	0.05712	0.05275
1.0	0.10349	0.09451	0.11214	0.10852	0.10467
1.2	0.21148	0.19412	0.20412	0.22041	0.20753
1.4	0.30992	0.30411	0.31145	0.32018	0.31142
1.6	0.42477	0.42114	0.40178	0.40921	0.41423
1.8	0.54335	0.55411	0.54122	0.53625	0.54373
2.0	0.66608	0.65122	0.67452	0.66421	0.66401
2.2	0.76421	0.78012	0.77842	0.77123	0.77659
2.4	0.87042	0.87712	0.86211	0.86412	0.86844
2.6	0.98452	1.0223	0.99122	0.98412	0.99554
2.8	0.99612	0.99587	0.99415	0.99621	0.99559
Correlation and regression coefficients $r = 0.9995$, $r^2 = 0.9990$ Standard error = 0.0530 Intercept = -0.4660 Slope = 0.5605					

**Figure 2**
Method linearity

The detection limit (LD) and the quantification limit (LQ) were calculated:

$$LD = \frac{3 \times 0.053}{0.5605} = 0.29 \mu\text{g/mL} \quad LQ = \frac{10 \times 0.053}{0.5605} = 0.95 \mu\text{g/mL}$$

Method precision: we calculated the sample concentration using the calibration curve equation (table IV). We observed that for each set of data and for both sets together the relative standard deviation was lower than 2% (RSD = 1.79%). This fact proved that the proposed method was precise.

Accuracy: the concentration of the samples was calculated from the experimental values of the absorbance, using the regression curve equation (table IV). We observed that the recovery was 100.89% for the studied concentration range, the mean (minimum was 98.45% and maximum was 103.22%) and the relative standard deviation was under 2% (RSD = 1.89). These values prove that the proposed method was accurate.

Table IV
Method precision and the accuracy

Meloxicam concentration µg/mL	Method precision		Intermediate precision		Accuracy	
	Absorbance	Recovery %	Absorbance	Recovery %	Absorbance	Recovery %
1.4	0.31142	99.07	0.31056	98.96	0.31420	99.43
	0.29452	96.92	0.34141	102.89	0.32652	101.00
	0.32892	101.30	0.29962	97.57	0.30985	98.87
1.6	0.41977	98.77	0.45041	102.19	0.45874	103.12
	0.43185	100.12	0.44041	101.07	0.44663	101.77
	0.44962	102.10	0.46085	103.35	0.45965	103.22
1.8	0.54335	100.04	0.56835	102.52	0.56847	102.53
	0.56122	101.82	0.55823	101.52	0.53956	99.67
	0.53023	98.74	0.54622	100.33	0.52729	98.45
Statistic	Mean = 99.88% RSD=1.69%		Mean = 101.16% RSD=1.89%		Mean = 100.89% RSD=1.80%	

Conclusions

A turbidimetric method was developed for the assay of meloxicam using molybdophosphoric acid in hydrochloric acid medium. The reaction product shows an absorbance maximum at 342 nm. The specific absorption coefficients of meloxicam in hydrochloric acid and of the reaction product were: $A_{1cm,339nm}^{1\%} = 722$ and $A_{1cm,342nm}^{1\%} = 3330$, respectively.

The analytical method was optimised and validated by establishing the linearity (in the range of 1.0 – 2.6 µg/mL), the correlation coefficient ($r = 0.9995$), the detection limit (0.29 µg/mL), the quantification limit (0.95 µg/mL), the method precision (RSD = 1.79 %) and the accuracy (mean recovery = 100.89 %).

In conclusion, the proposed method is simple, easy to perform, sensitive, linear, precise and accurate.

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