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ANALYSIS

COMPARISON OF CLASSIC AND DERIVATIVE UV SPECTROPHOTOMETRIC METHODS FOR QUANTIFICATION OF MELOXICAM AND MEFENAMIC ACID IN PHARMACEUTICAL PREPARATIONS

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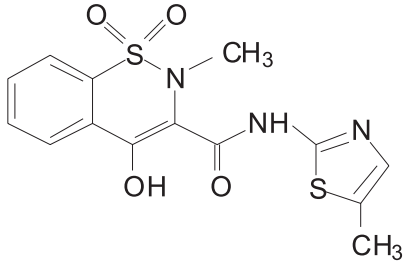
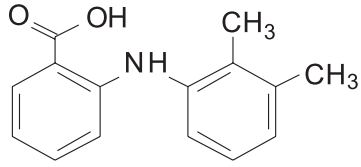
Abstract: The methods for quantitative determination of meloxicam and mefenamic acid in pharmaceuticals by classic spectrophotometry – zero order derivative, first and second order derivatives spectrophotometry is described, using „peak – peak" (P-P) and „peak – zero" (P-O) measurements. The calibration curves are linear within the concentration range of 4.0 – 14.0 μg/mL for meloxicam and 14.0 – 24.0 μg/mL for mefenamic acid. The procedure is simple, rapid and the results are reliable.

Keywords: meloxicam, mefenamic acid, classic spectrophotometry-zero order derivative; first and second order derivatives spectrophotometry

Meloxicam (Lutrol, Movalis, Metakam – 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide) and mefenamic acid (Mefacit, Parkemed, Ponalar, Ponstan, Ponstyl – 2-[(2,3-dimethylphenyl)amino]-benzoic acid) belong to the anti-inflammatory analgetics (1):



Meloxicam



Mefenamic acid

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the basic drugs used to treat rheumatoid arthritis. To this group, cyclooxygenase inhibitors, belongs oxicams with weak acid properties – enolic derivatives of the amide of benzothiazole-3-carboxylic acid, examples include meloxicam and derivatives of fenamic acid – mefenamic acid. The structural parameters of NSAIDs are still being explored.

The colorimetric method was used for determining flufenamic and mefenamic acids, ibuprofen, ketoprofen, diclofenac sodium and indomethacin in bulk and dosage forms after reaction with 2-nitrophenylhydrazine hydrochloride to give an intensive violet color at λ = 550 nm (2). The colorimetric method was also used for determination of mefenamic and enfenamic acids, ibuprofen, ketoprofen, diclofenac sodium and piroxicam after reaction with methylene violet at λ = 540 nm in pharmaceutical preparations (3). The spectrophotometric methods for the estimation of meloxicam in dosage forms is based on the formation of complex with ferric ammonium sulfate. The brownish green colored complex product is quantified at 396 nm (4). Spectrophotometric determinations of mefenamic acid in pharmaceutical preparations are based on the charge-transfer complexation between mefenamic acid and chloranil to form a violet chromogen measured at 540 nm (5) and on the reaction of mefenam-

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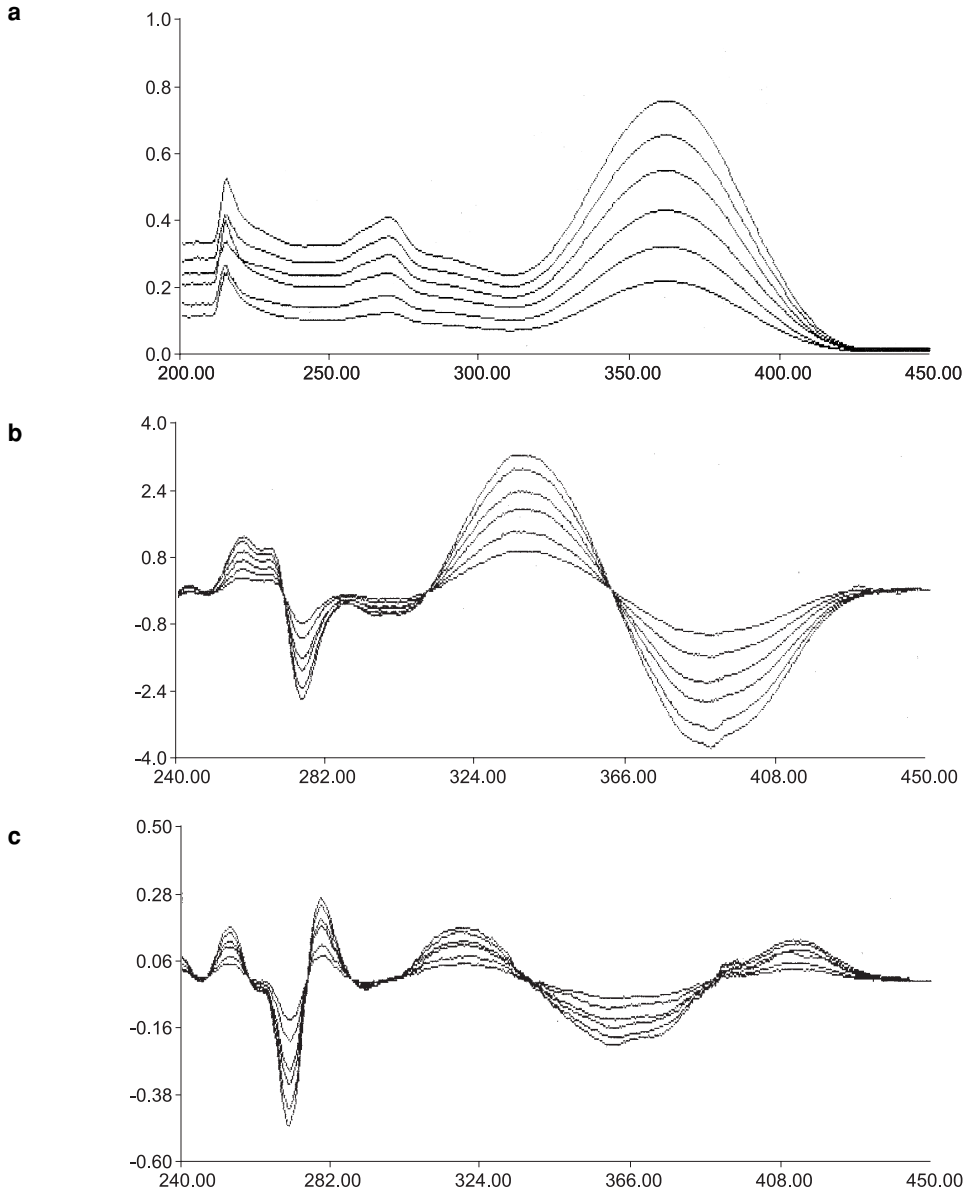

Figure 1. Zero- (**a**), first- (**b**) and second- (**c**) order derivative spectra of meloxicam standard solutions in 0.1 M NaOH in the range of 4.0 – 14.0 μg/mL

ic acid with: p-chloranilic acid to form a red color measured at 520 nm, N-bromosuccinamide measured at 362 nm, 3-methylbenzo-thiazolin-2-one hydrazone as a chromogenic reagent in the presence of ferric chloride solution measured at 602 nm (6). The spectrofluorimetric method was developed for determination of mefenamic acid in pharmaceutical preparation and human urine. This determination is based on the oxidation of mefenamic acid with cerium (IV) to produce cerium (III). Fluorescence was monitored at 354 nm after excitation at 255 nm (7). The spectrofluorimetric and the spectrophotometric stability-indicating methods have been developed for the determination of some oxicams (lornoxicam, tenoxicam and meloxicam) after derivatization of alkaline hydrolytic products with 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD-Cl). The products showed an absorption maximum at 460 nm for the three studied drugs and fluorescence emission peak at 535 nm in methanol (8).

This paper describes the application of the zero, first and second derivative UV-spectrophotometry for determination of meloxicam and mefenamic acid in pharmaceutical preparations.

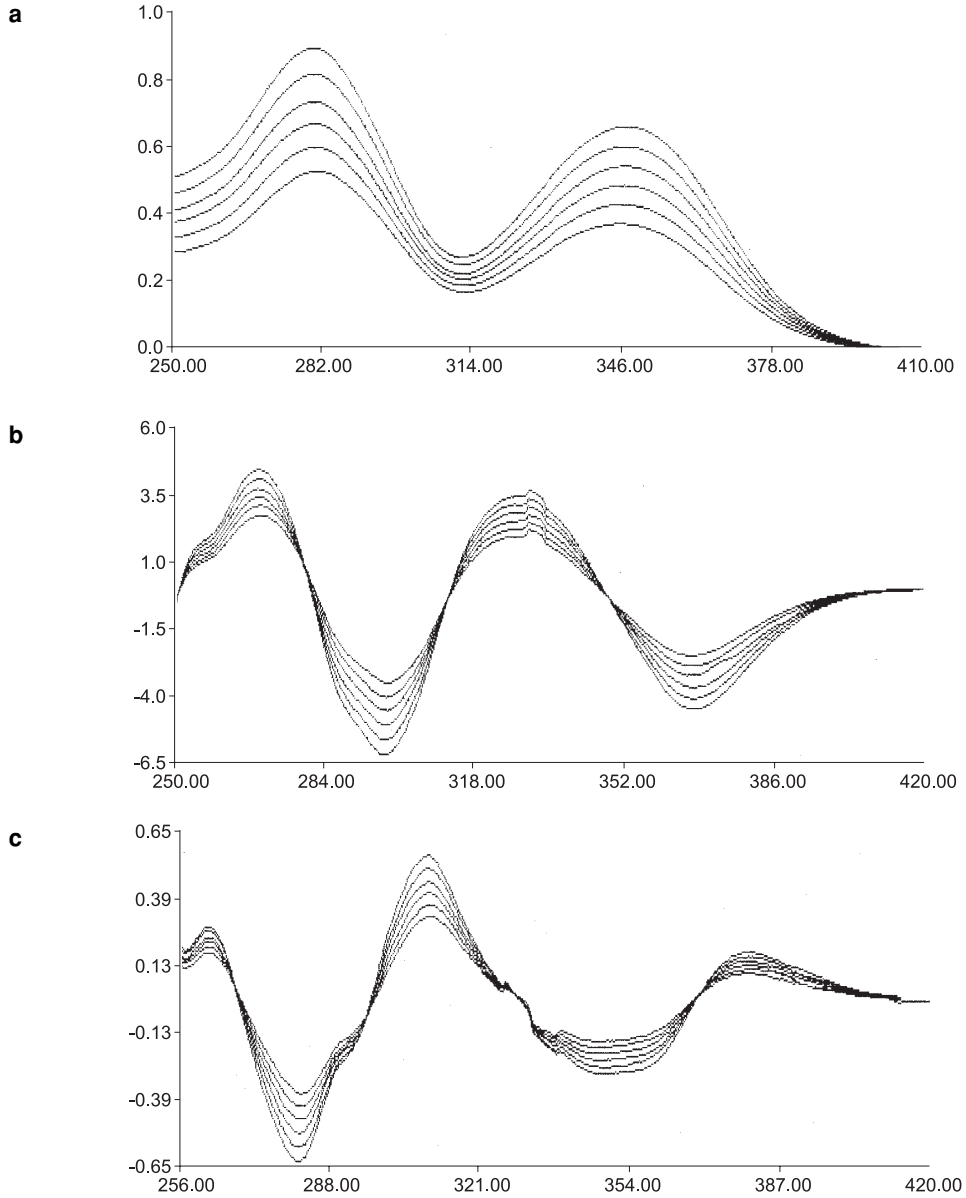

Figure 2. Zero- (**a**), first- (**b**) and second- (**c**) order derivative spectra of mefenamic acid standard solutions in methanol in the range of 14.0–24.0 μg/mL

EXPERIMENTAL

Reagents and apparatus

Meloxicam – substance was from Terpol S.A., Poland, mefenamic acid – substance was from Polfa S.A. Pabianice, Poland. Movalis – tablets 7.5 mg (Boehringer Ingelheim) and Mefacit – tablets 250 mg (Polfa S.A. Pabianice). A spectrophotometer, UV/VIS Perkin-Elmer, with the capability of applying the derivative mode was used. The optimized operating conditions for recording the zero-, first- and second- order derivative spectra were: scan speed 120.0 nm/min, response time 2 s, spectral slit width 2 nm. All measurements were carried out in 1.0 cm matched quartz cuvettes. Methanol and 0.1 M NaOH were used for dilutions.

Preparation of calibration curves for meloxicam and mefenamic acid

Stock solutions, 1.0 mg/mL, of meloxicam and mefenamic acid were prepared by dissolving appropiate amounts of meloxicam in 0.1 M NaOH and mefenamic acid in methanol. Working standard solutions of meloxicam and mefenamic acid con-

Table 1. Statistical evaluation of the elaborated method for meloxicam (standard solutions, n = 6).

Derivative	λ (nm)	Method	Regression equation	Correlation coefficient
D_0	361.2	P → O	$y = 0.0499\ (3.102\times10^{-3})\ x + 0.0093\ (1.040\times10^{-3})$	0.9999
D_0	270.0	P → O	$y = 0.0258\ (3.091\times10^{-3})\ x + 0.0065\ (1.108\times10^{-3})$	0.9928
D_0	215.6	P → O	$y = 0.0296\ (6.274\times10^{-3})\ x + 0.0718\ (3.412\times10^{-2})$	0.9936
D_1	386.0	P → O	$y = 0.249\ (2.817\times10^{-2})\ x + 0.0419\ (1.943\times10^{-2})$	0.9998
D_1	339.6	P → O	$y = 0.2149\ (2.993\times10^{-2})\ x + 0.0462\ (2.051\times10^{-2})$	0.9995
D_1	386.0–333.6	P → P	$y = 0.4639\ (3.624\times10^{-2})\ x + 0.0881\ (2.811\times10^{-2})$	0.9997
D_1	272.8	P → O	$y = 0.1547\ (3.205\times10^{-2})\ x + 0.0369\ (7.974\times10^{-2})$	0.9958
D_1	256.8	P → O	$y = 0.0855\ (3.915\times10^{-3})\ x + 0.0901\ (2.648\times10^{-2})$	0.9987
D_1	272.8–256.8	P → P	$y = 0.2403\ (2.703\times10^{-2})\ x + 0.127\ (0.910\times10^{-1})$	0.9987
D_2	409.2	P → O	$y = 0.0091\ (4.131\times10^{-4})\ x + 0.0016\ (2.537\times10^{-3})$	0.9992
D_2	359.2	P → O	$y = 0.014\ (3.638\times10^{-3})\ x - 0.002\ (3.227\times10^{-3})$	0.9994
D_2	409.2–359.2	P → P	$y = 0.0231\ (4.031\times10^{-3})\ x + 0.0035\ (2.293\times10^{-3})$	0.9994
D_2	315.6	P → O	$y = 0.0109\ (4.622\times10^{-3})\ x + 0.0055\ (5.008\times10^{-3})$	0.9966
D_2	359.2–315.6	P → P	$y = 0.0249\ (3.677\times10^{-3})\ x - 0.0074\ (1.713\times10^{-3})$	0.9996
D_2	277.6	P → O	$y = 0.0155\ (6.591\times10^{-3})\ x + 0.006\ (2.714\times10^{-3})$	0.9936
D_2	268.4	P → O	$y = 0.0272\ (3.082\times10^{-3})\ x + 0.0108\ (1.920\times10^{-2})$	0.9992
D_2	277.6–268.4	P → P	$y = 0.0426\ (5.402\times10^{-3})\ x - 0.0047\ (3.792\times10^{-3})$	0.9983
D_2	251.2	P → O	$y = 0.0097\ (2.791\times10^{-4})\ x - 0.0078\ (3.541\times10^{-3})$	0.9998
D_2	268.4–251.2	P → P	$y = 0.0368\ (3.382\times10^{-3})\ x - 0.0185\ (2.108\times10^{-2})$	0.9997

taining increasing concentrations of meloxicam (4.0 – 14.0 μg/mL) and mefenamic acid (14.0 – 24.0 μg/mL) were prepared from the stock solutions. The zero-, first- and second- order derivatives spectra of these solutions were recorded over the wavelength range 190 – 400 nm against the 0.1 M NaOH for meloxicam and methanol for mefenamic acid as blanks and the amplitudes of the maximum and minimum were measured.

Determination of meloxicam and mefenamic acid in tablets

Ten tablets of: movalis (a tablet, mean mass – 0.1818 g, contains 7.5 mg of meloxicam) and mefacit (a tablet, mean mass – 0.3434 g, contains 250 mg of mefenamic acid) were weighed and powdered. An accurately weighed portion of the powder (corresponding to 2.5 mg of meloxicam and 50 mg of mefenamic acid) were transferred into a 25 mL

Table 2. Statistical evaluation of the elaborated method for mefenamic acid (standard solutions. n = 6)

Derivative	λ (nm)	Method	Regression equation	Correlation coefficient
D_0	81.2	P → O	$y = 0.0366\ (1.806\times10^{-3})\ x + 0.0098\ (2.460\times10^{-3})$	0.9998
D_1	298.0	P → O	$y = 0.2554\ (1.908\times10^{-2})\ x - 0.0087\ (2.264\times10^{-2})$	0.9998
D_1	269.2	P → O	$y = 0.1626\ (2.117\times10^{-2})\ x + 0.4108\ (2.931\times10^{-2})$	0.9994
D_1	298–269	P → P	$y = 0.418\ (5.020\times10^{-3})\ x + 0.4008\ (3.871\times10^{-2})$	0.9997
D_2	380.4	P → O	$y = 0.0078\ (5.147\times10^{-4})\ x - 0.0071\ (4.861\times10^{-3})$	0.9991
D_2	348.0	P → O	$y = 0.0122\ (8.443\times10^{-3})\ x - 0.0086\ (6.801\times10^{-3})$	0.9989
D_2	380.4–348.0	P → P	$y = 0.02\ (6.779\times10^{-3})\ x - 0.017\ (3.276\times10^{-3})$	0.9991
D_2	310.0	P → O	$y = 0.0231\ (2.017\times10^{-4})\ x - 0.0022\ (1.038\times10^{-3})$	0.9999
D_2	348.0–310.0	P → P	$y = 0.0353\ (3.272\times10^{-4})\ x - 0.0103\ (1.980\times10^{-3})$	0.9998
D_2	282.0	P → O	$y = 0.0258\ (2.851\times10^{-4})\ x - 0.0008\ (1.379\times10^{-5})$	0.9999
D_2	310.0–282.0	P → P	$y = 0.0489\ (3.164\times10^{-4})\ x - 0.0015\ (2.835\times10^{-4})$	0.9999
D_2	262.4	P → O	$y = 0.01050\ (5.712\times10^{-3})\ x + 0.0321\ (8.036\times10^{-3})$	0.9970
D_2	292.0–262.4	P → P	$y = 0.0363\ (1.244\times10^{-3})\ x + 0.0322\ (5.070\times10^{-3})$	0.9998

volumetric flasks containing approximately 15 mL of 0.1 M NaOH for determination of meloxicam or 15 mL of methanol for determination of mefenamic acid. The mixtures were shaked for 30 min, then the volumes were brought to 25.0 mL with 0.1 M NaOH or methanol, respectively, and the solutions obtained were filtered. After filtration, meloxicam and mefenamic acid solutions of 1.0 mL volume were transferred into a 10 mL volumetric flasks and made up to 10 mL with 0.1 M NaOH or methanol, respectively. Next, 1.0 mL volume of mefenamic acid was transferred into a 10 mL volume flask and made up to 10.0 mL with methanol.

The zero-, first- and second- order derivative spectra of final solutions were recordered.

RESULTS AND DISCUSSION

In initial tests assays of the stability of the analysed compounds in 0.1 M NaOH and methanol were performed by the spectrophotometric method. The best results for the analytical purposes were obtained with 0.1 M NaOH for meloxicam and methanol for mefenamic acid. The solutions used were stable for one month. In order to determine the values of derivative spectra, two graphical techniques: „peak – zero" and „peak – peak" have been used. In the „peak – peak" technique the determination was carried out by measuring the amplitude from a maximum to a minimum of the curve. In the „baseline to peak" technique, the measurement was carried out from a maximum to the zero line or from a minimum to the zero line. The calibration curves were constructed by plotting the graphically measured (nm) amplitudes of the zero-, first- and second-order derivatives spectra *vs*. the corresponding concentrations of the examined drugs. The meloxicam content was determined for the zero derivative at 270.0 nm; for the first derivative at 339.6 nm; for the second derivative at 315.6 nm at the „peak – zero". The mefenamic acid was determined for the zero derivative at 281.2 nm at the „peak – zero"; for the first derivative at 298.0–269.2 nm; for the second derivative at 310.0–282.0 nm at the „peak – peak".

Table 3. Statistical analysis of the determination of meloxicam in tablets „Movalis" 7.5 mg

Derivative	λ (nm)	Method	x (mg)	SD	SD x	RSD (%)	$U_{95\%}$	$E_{rel.}$ (%)
D_0	361.2	P→O	7.24	0.09	0.04	1.22	±0.11	–3.52
D_0	270.0	P→O	7.38	0.31	0.14	4.17	±0.38	–1.55
D_0	215.6	P→O	12.04	0.66	0.29	5.48	±0.82	60.48
D_1	386.0	P→O	7.17	0.09	0.04	1.29	±0.11	–4.46
D_1	339.6	P→O	7.31	0.11	0.05	1.53	±0.14	–2.53
D_1	386.0–333.6	P→P	7.21	0.13	0.60	1.86	±0.17	–3.89
D_1	272.8	P→O	7.19	0.25	0.11	3.43	±0.30	–4.10
D_1	256.8	P→O	7.16	0.29	0.13	4.06	±0.36	–4.51
D_1	272.8–256.8	P→P	7.20	0.24	0.11	3.39	±0.30	–3.97
D_2	409.2	P→O	7.19	0.11	0.05	1.49	±0.13	–4.11
D_2	359.2	P→O	7.24	0.10	0.04	1.37	±0.12	–3.49
D_2	409.2–359.2	P→P	7.22	0.10	0.04	1.41	±0.13	–3.68
D_2	315.6	P→O	7.47	0.28	0.12	3.71	±0.34	–0.45
D_2	359.2–315.6	P→P	7.34	0.16	0.07	2.13	±0.19	–2.08
D_2	277.6	P→O	6.67	0.16	0.07	2.43	±0.20	–11.04
D_2	268.4	P→O	7.18	0.10	0.04	1.42	±0.13	–4.24
D_2	251.2	P→O	7.69	0.17	0.07	2.18	±0.21	2.61
D_2	268.4–251.2	P→P	7.33	0.11	0.05	1.46	±0.13	–2.21

Table 4. Statistical analysis of the determination of mefenamic acid in tablets „Mefacit" 250 mg

Derivative	λ (nm)	Method	x (mg)	SD	SD x	RSD (%)	$U_{95\%}$	$E_{rel.}$ (%)
D_0	281.2	P→O	243.10	3.35	1.49	1.38	± 4.16	–2.76
D_1	298.0	P→O	244.19	3.65	1.63	1.49	± 4.53	–2.32
D_1	269.2	P→O	244.66	3.49	1.56	1.42	± 4.33	–2.13
D_1	298.0–269.2	P→P	244.93	2.81	1.25	1.15	± 3.49	–2.03
D_2	380.4	P→O	219.74	3.63	1.62	1.65	± 4.51	–12.10
D_2	348.0	P→O	226.10	3.55	1.59	1.57	± 4.41	–9.56
D_2	380.4–348.0	P→P	225.24	5.42	2.42	2.40	± 6.73	–9.90
D_2	310.0	P→O	242.81	4.13	1.85	1.70	± 5.13	–2.88
D_2	348.0–310.0	P→P	237.21	3.87	1.73	1.63	± 4.80	–5.12
D_2	282.0	P→O	243.13	3.20	1.43	1.311	± 3.97	–2.75
D_2	310.0–282.0	P→P	243.35	3.61	1.61	1.48	± 4.48	–2.66
D_2	264.4	P→O	240.77	6.87	3.07	2.85	± 8.53	8.53
D_2	282.0–262.4	P→P	242.68	3.54	1.58	1.46	± 4.39	4.39

The procedure was repeated six times. The values were compared with an appropriate calibration graph. Figures 1 and 2 show the zero-, first- and second- order derivative spectra of meloxicam and mefenamic acid respective standard solutions in 0.1 M NaOH for meloxicam and methanol for mefenamic acid, recordered within the range of 190–400 nm at a concentration of 10.0 mg/mL of meloxicam

Table 5. Comparison of the elaborated methods used to the determination of meloxicam and mefenamic acid in pharmaceutical preparations (I – D_0. II – D_1. III – D_2).

Method	Pharmaceutical form (active substance)	
	Movalis (meloxicam)	Mefacit (mefenamic acid)
	F- Snedecor´s test. $\alpha = 95\%$; $F_{\alpha(4.4)} = 6.390$ *t*-Student´s test. $\alpha = 95\%$; $t_{\alpha 4} = 2.776$	
I ⇔ II	$F_{calculated} = 7.942$ $t_{calculated} = 0.4759$	$F_{calculated} = 1.421$ $t_{calculated} = 0.9359$
I ⇔ III	$F_{calculated} = 1.226$ $t_{calculated} = 0.4818$	$F_{calculated} = 1.161$ $t_{calculated} = 0.1135$
II ⇔ III	$F_{calculated} = 6.379$ $t_{calculated} = 1.189$	$F_{calculated} = 1.650$ $t_{calculated} = 0.7722$

and 20.0 μg/mL of mefenamic acid. Detection and quantification limits were: 1.30 μg/mL and 3.50 μg/mL for D_0 (λ = 270.0 nm), 1.00 μg/mL and 3.50 μg/mL for D_1 (λ = 339.6 nm), 1.20 μg/mL and 3.80 μg/mL for D_2 (λ = 315.6 nm), for meloxicam. For mefenamic acid, detection and quantitation limits were: 5.60 μg/mL and 14.0 μg/mL for D_0 (λ = 281.2 nm), 4.90 μg/mL and 12.6 μg/mL for D_1 (λ = 298.0–269.2 nm), 4.55 μg/mL and 12.6 μg/mL for D_2 (λ = 310.0–282.0 nm), respectively. The linear equations obtained through regression analysis of the data on meloxicam and mefenamic acid are shown in Tables 1 and 2. The indispensable time necessary for the extraction of both drugs from tablets was 30 min. Tables 3 and 4 illustrate the data on the determination of meloxicam and mefenamic acid in tablets with statistical evaluation of the results. The best result for determining meloxicam in Movalis 7.5 mg tablets was obtained by the „peak – zero" technique for the zero derivative spectrum at a wavelength of 270.0 nm (± SD 0.31; RSD 4.17%; recovery 98.45%); for the first derivative spectrum at wavelength of 339.6 nm (± SD 0.11; RSD 1.53%; recovery 97.47%) and for the second derivative spectrum at wavelength of 315.6 nm (± SD 0.28; RSD 3.71%; recovery 99.55%). The best result for determining mefenamic acid in Mefacit 250 mg tablets was obtained by the „peak – zero" technique for the zero derivative spectrum at a wavelength of 281.2 nm (± SD 3.35; RSD 1.38%; recovery 97.24%); by the „peak – peak" technique for the first derivative spectrum at wavelengths of 298.0–269.2 nm (± SD 2.81; RSD 1.15%; recovery 97.97%) and for the second derivative spectrum at wavelengths of 310.0–282.0 nm (± SD 3.61; RSD 1.48%; recovery 97.34%).

F-Snedecor´s test and Student´s *t*-test (Table 5) showed no significant difference (except for D_0 ⇔ D_1 method for Movalis $F_{calculated} = 7.942$) between the mean recovery and 100% (95%, F calculated and *t* calculated for Movalis and for Mefacit was less than the tabulated value of F and *t*, respectively).

In summary, the proposed analytical procedure based on the second-order derivative at a wavelength of 315.6 nm for meloxicam (recovery 99.55%) and on the first derivative at wavelengths of 298.0–269.2 nm for mefenamic acid (recovery 97.97%) spectroscopy permits a simple, rapid, sensitive and direct determination of the analysed drugs.

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