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Densitometric determination of diclofenac, 1-(2,6-dichlorophenyl)indolin-2-one and indolin-2-one in pharmaceutical preparations and model solutions

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Abstract

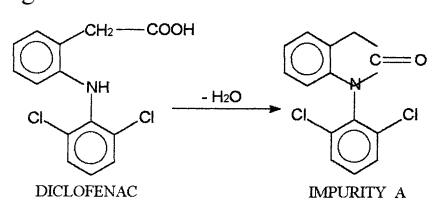
A chromatographic-densitometric method for identification and quantitation of diclofenac and its impurities, i.e. 1-(2,6-dichlorophenyl)indolin-2-one and indolin-2-one in pharmaceutical preparations and model solutions was developed. The effect of pH, temperature and ultra violet (UV) radiation on diclofenac's concentration was investigated. Chromatographic separation was performed on TLC silica gel coated plates with the mobile phase: cyclohexane–chloroform–methanol (12:6:1, v/v/v). Densitometric detection was carried out in UV at $\lambda = 248$ nm. The conditions for good separation and the detection limit were established. The recovery for diclofenac was 99.20%, for 1-(2,6-dichlorophenyl)indolin-2-one—92.34% and for indolin-2-one—95.85%. The method was used for quality assessment of diclofenac in pharmaceutical preparations. Reliable results comparable to those determined by high performance liquid chromatography (HPLC) were obtained.

Keywords: Analysis of pharmaceuticals; Densitometry; TLC; Impurity; Diclofenac; 1-(2,6-dichlorophenyl)indolin-2-one; Indolin-2-one

1. Introduction

Diclofenac belongs to benzeneacetic acid derivatives and it is a sodium salt of 2-[(2,6-dichlorophenyl)-amino-phenyl]acetic acid [1]. It is commonly used as a strong analgesic and anti-inflammatory agent in various drug formulations such as injections, suppositories and ointments [3].

Stability analysis has revealed that diclofenac [4,5] in acid solutions undergoes cyclization with a removal of a water molecule according to the following reaction: .



DICLOFENAC IMPURITY A

The reaction produces 1-(2,6-dichlorophenyl)-indolin-2-one, listed by the European Pharmaco-

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poeia [1] and by the British Pharmacopoeia [2] as one of five potential impurities (imp. A) that should be taken into account in drug quality assessment.

Available papers on determination of diclofenac and its potential impurities in common medicines were reviewed. Most authors recommend spectrophotometric methods for determination of diclofenac and other constituents originated from the matrix or for the analysis of complex drugs [6–10].

Spectrofluorometric methods [11], X-ray fluorescence analysis [12,13], gas chromatography [14,15] and high performance liquid chromatography (HPLC) [16,17] were also used in the analysis of drugs containing diclofenac.

In this paper a new method for simultaneous determination of diclofenac, 1-(2,6-dichlorophenyl)indolin-2-one (imp. A) and indolin-2-one (imp. E) in various drug forms was developed. The method was used for the analysis of drug stability in aqueous solutions, depending on pH, temperature and ultra violet (UV) radiation. This study is based on previous successful applications [18–22] of densitometric method that can be an alternative to commonly used HPLC.

2. Experimental

2.1. Apparatus

1. Densitometer TLC Scanner 3 with Cats4 software, manufacturer—CAMAG (Muthenz, Switzerland).
2. Sample applicator—Linomat IV, manufacturer as above.
3. Computer—PC Pentium MMX, 16 MB RAM (Taiwan) and HP LaserJet 6L Printer (USA).
4. TLC Plates—9 × 15 cm (cut from 20 × 20 cm precoated silica gel aluminium TLC sheets Art. No. 16484, supplier, E. Merck—Darmstadt, Germany).
5. TLC chamber of 18 × 9 × 18 cm in size, manufacturer, Sigma-Aldrich.
6. Liquid Chromatograf LaChrom (Merck–Hitachi) with detector DAD and automatic sample feeder, computer controlled by the D-7000HSM software.
7. Filter, millipore corporation, Bedford, MA 01730, pore size 0.45 μM.

2.2. Preparations examined

1. Majamil, tablets containing 50 mg of diclofenac; s. 1150101 exp. 012003 (Polpharma, Poland).
2. Diclofenac Sodium, tablets containing 50 mg of diclofenac; s. BNCAH43A exp. EXP0700 (Norton, UK).
3. Rewodina retard, tablets containing 100 mg of diclofenac; s. 8004.1 exp. 11.2000 (AWD, Germany).
4. Voltaren, injections containing 75 mg of diclofenac; s. MFD021999 exp. EXP022001 (Novartis, Switzerland).
5. Olfen, injections containing 75 mg of diclofenac and 20 mg lidokaine; s. 9901404 exp. 11.2003 (mepha, Switzerland).
6. Artotec, tablets containing 50 mg of diclofenac and 200 μg misoprostol; s. 856720 exp. 01.2001 (Monsanto, France).
7. Dicloratio Retard, capsules containing 100 mg of diclofenac; s. 938117 exp. 08.2000 (Ratiopharm, Germany).
8. Majamil, tablets containing 25 mg of diclofenac; s. 50898 exp. 082000 (Polpharma, Poland).

2.3. Reagents and chemicals

1. Standard substances: diclofenac (STADA); 1-(2,6-dichlorophenyl)indolin-2-one (Polpharma); indolin-2-one (Fluka).
2. Reagents: methanol (Merck); cyclohexane, chloroform (POCH Gliwice, Poland); phosphoric acid (Park, UK); sodium dihydrogen phosphate (POCH Gliwice, Poland).

2.4. Solutions for densitometric analysis

1. Standard solutions: diclofenac 0.026%, w/v, 1-(2,6-dichlorophenyl)indolin-2-one (imp. A) 0.02%, w/v and indolin-2-one (imp. E) 0.01%,

w/v. Solutions were prepared in methanol–water (1:1, v/v).

2. Sample solutions:
   - tablets and capsules—ten tablets were powdered in a mortar. Thoroughly powdered tablets containing approximately 20.0 mg of diclofenac were added to 20.0 ml of methanol–water (1:1, v/v) and shaken for 30 min. The resulting suspension was filtered and used for further analysis.
   - Injections—a volume of solution corresponding to 20.0 mg of diclofenac was weighed and diluted with methanol–water (1:1, v/v) to 20.0 ml.

2.5. Solutions for HPLC analysis

1. Mobile phase: a mixture of 34 volumes of a mixture of equal volumes of a 1 g/l solution of phosphoric acid and a 1.6 g/l solution of sodium dihydrogen phosphate adjusted to pH 2.5, and of 66 volumes of methanol.
2. Impurity A standard solution: 0.002%, v/v in mobile phase.
3. Diclofenac standard solution: 0.005%, v/v in mobile phase.
4. Sample solutions for examination: an amount of preparation containing 50 mg of diclofenac was weighed, shaken for 30 min in 70.0 ml of

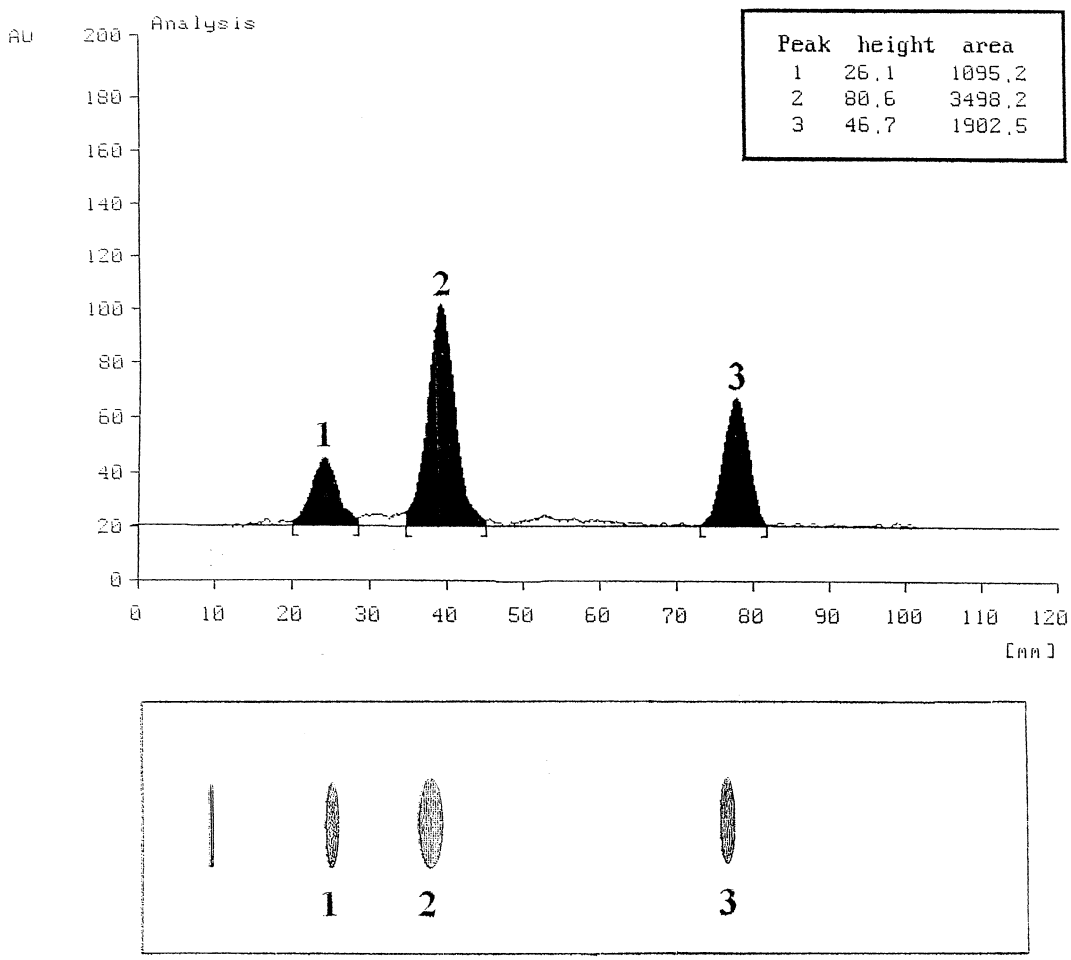


Fig. 1. An example of densitogram and chromatogram of dileofenae (**2**), 1-2(2,6-dichlorophenyl)indolin-2-one (**3**) and indolin-2-one (**1**).

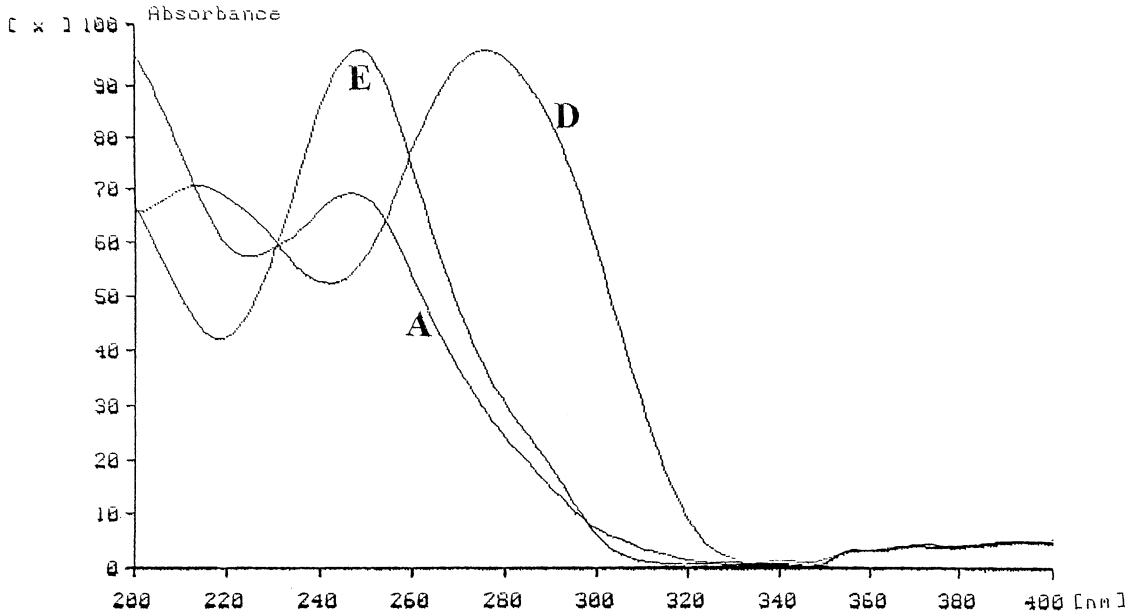


Fig. 2. Absorption spectra for diclofenac (**D**), 1-(2,6-dichloropheny)indolin-2-one (**A**) and indolin-2-one (**E**) recorded from chromatogram.

mobile phase, an than topped up to 100.0 ml with the same solution. For tablets, the resulting suspension was filtered through the millipore filter of 0.45 μm.

3. Results

In further investigations the procedure was validated to establish the conditions for determina-

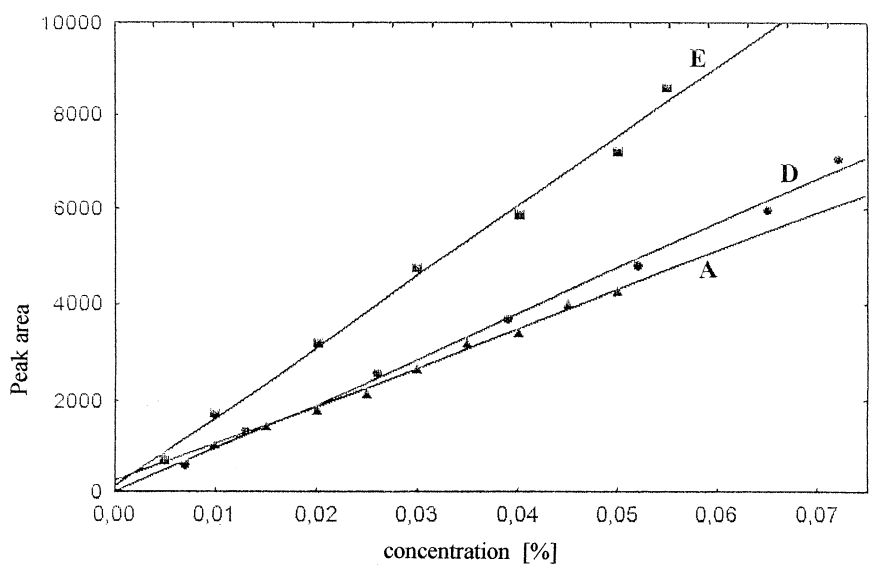


Fig. 3. The relationship between peak area and concentration of, diclofenac (**D**; $P_{\mathbf{D}} = 10.258 + 94791 \times c_D$), 1-(2,6-dichloropheny)indolin-2-one (**A**; $P_{\mathbf{A}} = 245.96 + 81007 \times c_A$) and indolin-2-one (**E**; $P_{\mathbf{E}} = 122.97 + 148600 \times c_E$).

tion of diclofenac and its potential impurities in pharmaceutical preparations [23].

The analysis was carried out by two analytical chemists and the influence of previously planned little changes of parameters on the results was taken into account. The same apparatus and reagents were used.

3.1. Robustness (the effect of parameter changes on measurements)

To establish the optimal separation conditions, the amounts ranging from 1 to 10 μl of standard solutions were applied in the form of 1 cm bands on the chromatographic plates of 9×15 cm. Chromatograms were developed in various mobile phases over different distances. A good separation of constituents was reached over the mobile phase distance of 12 cm in a relatively short time of about 60 min.

The UV densitometry was used for recording the chromatogram spots. The values of R_f for individual constituents were determined from densitograms.

The use of mobile phase, cyclohexane–chloroform–methanol (12:6:1, v/v/v) allows to obtain chromatograms with well developed and separated peaks (Fig. 1).

The absorption spectra within the range from 200 to 400 nm recorded for each constituent are presented in Fig. 2.

In the analysed absorption spectrum diclofenac shows a maximum at the wavelength $\lambda = 280$ nm, whereas the impurities at $\lambda = 248$ nm. The latter

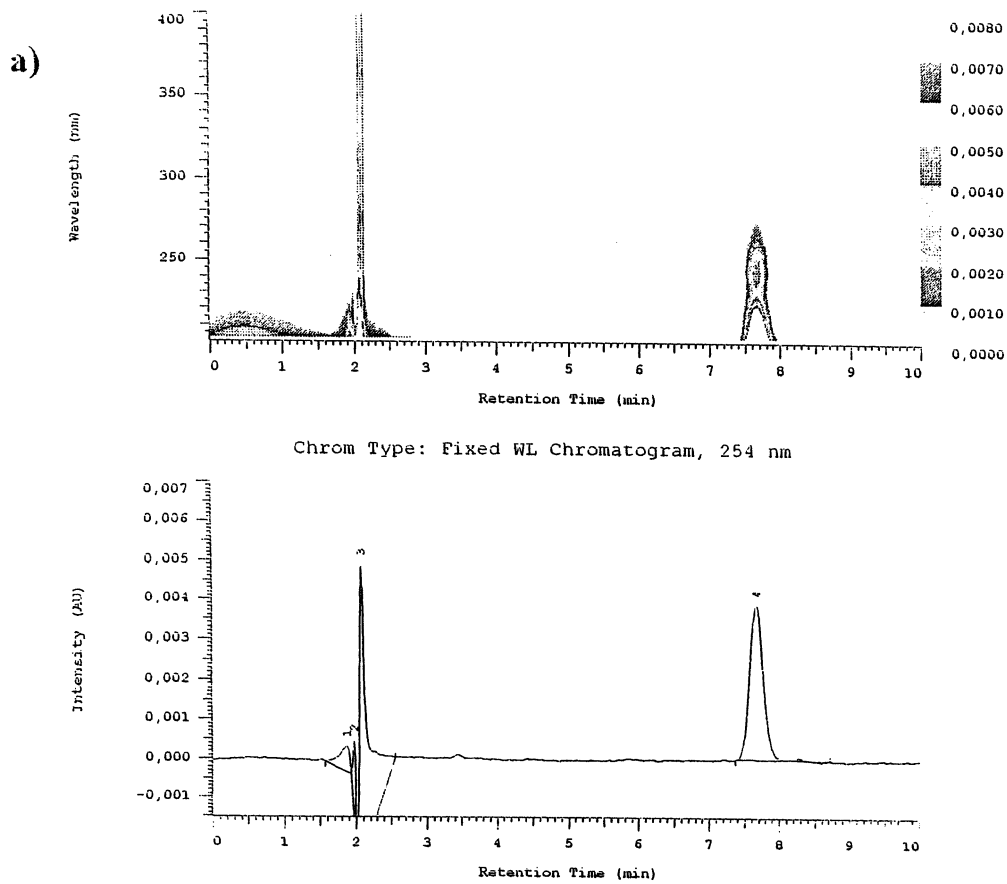


Fig. 4. Chromatograms obtained by HPLC, standard impurity A (a) and analysed sample (b).

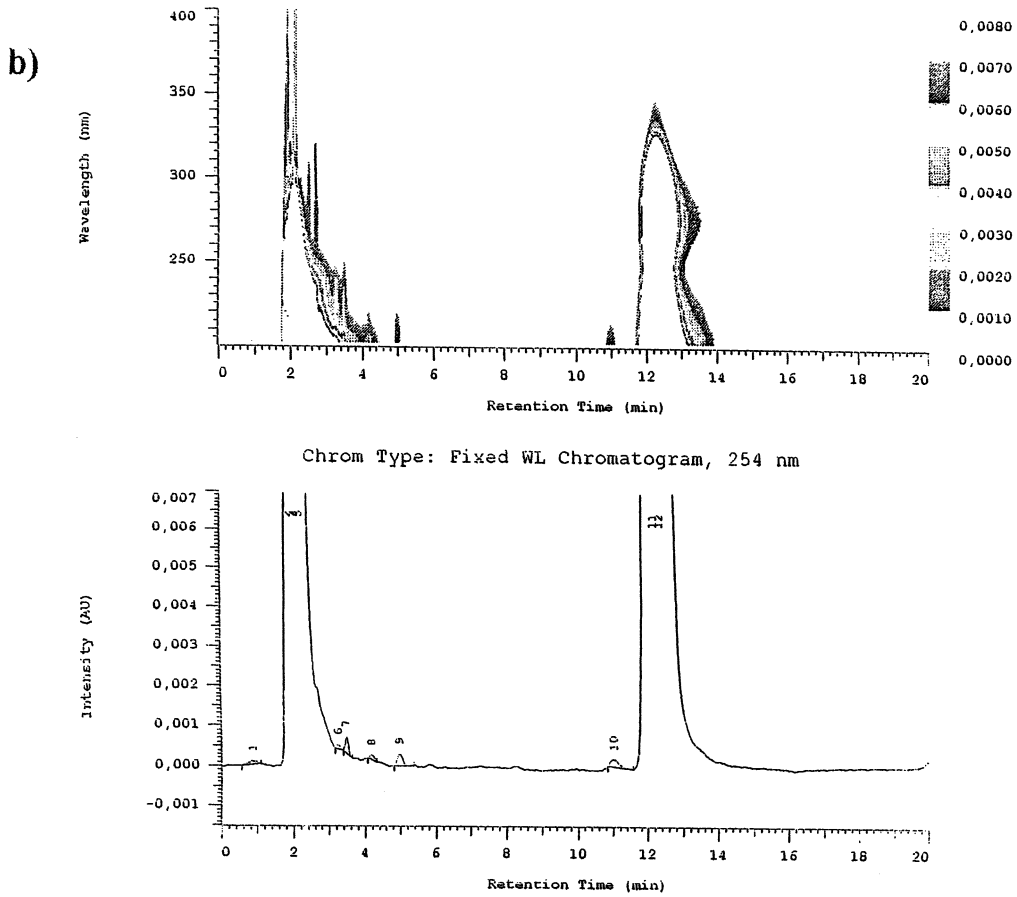


Fig. 4. (*Continued*)

wavelength was chosen to determine all constituents because it allows to carry out research simultaneously and makes the analysis faster.

3.2. Linearity

For a linearity study seven solutions of different concentration ranging from 0.007 to 0.072%, v/v for diclofenac, from 0.005 to 0.050%, v/v for impurity A and from 0.005 to 0.055%, v/v for impurity E were prepared. The linear regression method was employed (Fig. 3).

3.3. Quantitation limit and detection limit

The detection limit and the determination limit were considered together, as under the established chromatographic conditions well developed peaks with a negligible noise level were obtained. Decreasing concentrations of the analysed constituents were added to the preparation examined while recording the peak areas on the chromatograms. The limit of detection was determined from the signal of S/N ratio equal to at least 4. The background was established from a 'blind' sample. The following limits of detection were found: 0.13 μg for diclofenac, 0.20 μg for impurity A and 0.05 μg for impurity E.

3.4. Selectivity

Since the aim of this study was to develop a method for determining the active substance and its potential impurities in various drug forms, the

Table 1
The results of diclofenac determination in preparations with statistical analysis

Preparation/declared concentration of diclofenac	Determined concentration of diclofenac (%, w/w)			Statistical analysis ($n = 7$)
Majamil/50 mg in tablets	95.80	96.40		$\times = 98.69$
	106.40	102.20	$S = 4.69$	$S_{\times} = 1.77$
	93.60	101.40		$\mu = 98.69 \pm 4.33$
	95.00			R.S.D. = 4.75
Diclofenac sodium/50 mg in tablets	99.40	103.40		$\times = 100.86$
	103.20	99.40	$S = 1.92$	$S_{\times} = 0.73$
	100.00	98.80		$\mu = 100.86 \pm 1.79$
	101.80			R.S.D. = 1.90
Rewodina retard/100 mg in tablets	102.40	98.90		$\times = 99.19$
	100.60	97.30	$S = 2.29$	$S_{\times} = 0.87$
	98.50	95.70		$\mu = 99.19 \pm 2.13$
	100.90			R.S.D. = 2.31
Voltaren/75 mg in injections	102.13	98.00		$\times = 100.80$
	99.87	99.73	$S = 2.57$	$S_{\times} = 0.97$
	101.87	105.47		$\mu = 100.80 \pm 2.37$
	98.53			R.S.D. = 2.55
Olfen/75 mg in injections	98.67	104.00		$\times = 100.72$
	99.87	100.40	$S = 1.82$	$S_{\times} = 0.69$
	101.73	99.07		$\mu = 100.72 \pm 1.69$
	101.33			R.S.D. = 1.81
Artotec/50 mg in tablets	95.80	98.40		$\times = 99.91$
	100.60	98.60	$S = 3.25$	$S_{\times} = 1.23$
	103.00	97.80		$\mu = 99.91 \pm 3.01$
	105.20			R.S.D. = 3.25
Dicloratio retard/100 mg in capsules	96.30	101.20		$\times = 101.09$
	105.20	101.00	$S = 4.12$	$S_{\times} = 1.56$
	100.00	96.50		$\mu = 101.09 \pm 3.82$
	107.40			R.S.D. = 4.08
Majamil/25 mg in tablets	99.20	96.80		$\times = 100.11$
	103.60	97.60	$S = 3.52$	$S_{\times} = 1.33$
	96.00	104.00		$\mu = 100.11 \pm 3.25$
	103.6			R.S.D. = 3.52

$\times$, arithmetic mean; S, standard deviation for individual points; $S_{\times}$, standard deviation for arithmetic mean; μ, confidence interval at 95% probability; R.S.D., relative standard deviation (%).

effect of potential impurities originated both from the matrix and from coexisting active substances was analysed. For this purpose model solutions containing comparative substances present in Artotec 50 and Olfen 75 and appropriate solutions for examination at concentrations identical to those of model solutions were prepared.

The solutions were separated chromatographically and the absorption spectra and peak areas were recorded. Similar absorption spectra and peak areas were obtained. It was found that additional spots produced by coexisting substances have different R_f values (lidocaine $R_f \approx 0.13$, misoprostol $R_f \approx 0.00$, diclofenac $R_f \approx 0.32$), than the impurities under investigation (imp. A $R_f \approx 0.67$, imp. E $R_f \approx 0.21$).

Thus, it was assumed that both the coexisting substances and potential impurities from the matrix had no effect on the determination of diclofenac and impurities A and E.

Table 2
The results of diclofenac and 1-(2,6-dichlorophenyl)indolin-2-one (impurity A) determination for various concentrations of HCl at temperature 22 °C

Preparation	c (HCl) (mol/dm^3)	0 day		5 days		13 days		20 days		43 days	
		i.A	**D**	i.A	**D**	i.A	**D**	i.A	**D**	i.A	**D**
Olfen	1.000	0.00	34.70	8.49	8.26	11.31	7.02	7.77	4.91	32.90	4.48
	0.500	0.00	42.75	5.86	9.11	7.69	8.55	7.70	7.28	22.48	5.30
	0.100	0.00	41.81	1.93	22.27	2.03	20.23	5.11	15.41	8.89	11.69
	0.050	0.00	42.25	0.83	29.93	0.92	25.87	2.65	20.70	5.54	12.59
	0.020	0.00	69.92	0.00	29.11	0.00	26.32	0.66	26.44	1.97	21.66
	0.010	0.00	82.68	0.00	62.16	0.00	31.47	0.00	27.17	0.26	24.26
	0.001	0.00	90.61	0.00	84.37	0.00	80.53	0.00	79.18	0.00	71.33
Dicloratio retard	1.000	0.00	21.07	5.45	5.71	8.72	2.92	11.24	2.24	9.84	0.00
	0.500	0.00	23.48	3.16	5.82	4.96	5.00	6.66	3.84	6.31	1.95
	0.100	0.00	26.01	0.94	11.28	1.50	7.51	3.25	7.25	3.61	5.75
	0.050	0.00	33.86	0.58	23.59	1.30	12.86	2.00	10.89	2.60	8.14
	0.020	0.00	43.80	0.00	43.74	0.79	25.39	0.84	21.79	1.61	16.72
	0.010	0.00	61.23	0.00	65.68	0.00	51.28	0.00	39.69	1.12	23.55
	0.001	0.00	85.41	0.00	98.62	0.00	101.59	0.00	102.22	0.00	90.43
Majamil	1.000	1.39	11.75	6.51	10.35	11.45	2.50	15.75	0.00	15.06	0.00
	0.500	1.54	27.43	7.51	17.95	10.83	6.57	9.30	6.88	19.52	5.56
	0.100	0.57	30.96	1.21	25.88	3.33	24.17	5.20	12.06	8.99	6.04
	0.050	0.00	32.95	0.60	26.78	1.05	23.75	1.69	21.76	5.27	15.49
	0.020	0.00	33.19	0.00	33.40	0.00	35.17	0.84	30.31	1.85	17.11
	0.010	0.00	28.97	0.00	29.03	0.00	29.54	0.00	29.74	0.00	24.09
	0.001	0.00	81.16	0.00	85.14	0.00	83.18	0.00	79.60	0.00	67.32

i.A, % (w/w) concentration of impurity A with reference to active substance (diclofenac); **D**, diclofenac concentration (%, w/w).

Table 3
The results of diclofenac and 1-(2,6-dichlorophenyl)indolin-2-one (impurity A) determination for various concentrations of HCl at temperature 37 °C

Preparation	c (HCl) (mol/dm^3)	0 day		1 day		3 days		4 days		7 days		9 days		14 days	
		i.A	**D**	i.A	**D**	i.A	**D**	i.A	**D**	i.A	**D**	i.A	**D**	i.A	**D**
Olfen	1.000	0.00	34.70	10.73	24.78	13.01	10.34	33.37	11.23	43.15	5.99	45.72	5.30	49.18	0.00
	0.500	0.00	42.75	6.77	33.28	13.02	16.95	22.70	17.34	32.33	18.54	37.69	12.90	50.17	7.59
	0.100	0.00	41.81	2.34	44.42	6.36	35.27	8.06	26.35	12.27	27.64	13.90	17.69	20.19	16.76
	0.050	0.00	42.25	0.95	48.72	3.72	33.37	2.78	31.71	6.35	31.71	8.17	30.31	11.59	26.09
	0.020	0.00	69.92	0.00	48.33	2.88	41.95	1.53	43.02	2.53	42.80	3.19	35.47	6.32	34.35
	0.010	0.00	82.68	0.00	72.41	0.00	57.84	0.00	58.15	0.27	57.06	0.47	53.73	0.73	51.18
	0.001	0.00	90.61	0.00	90.73	0.00	79.66	0.00	77.79	0.00	78.49	0.00	80.69	0.00	76.29
Dicloratio retard	1.000	0.00	21.07	4.73	6.04	9.96	3.93	24.20	2.63	16.29	1.50	14.85	1.01	14.16	0.00
	0.500	0.00	23.48	2.89	7.69	6.92	5.09	7.92	4.74	8.80	3.15	8.55	2.33	10.79	0.00
	0.100	0.00	26.01	0.78	15.03	1.78	10.53	2.23	8.41	2.73	8.91	3.84	7.38	5.26	6.67
	0.050	0.00	33.86	0.00	25.44	1.66	14.93	1.93	13.65	2.36	10.52	2.88	9.58	4.67	10.22
	0.020	0.00	43.80	0.00	38.89	1.07	31.37	1.35	30.85	1.46	24.56	1.85	25.60	3.18	21.81
	0.010	0.00	61.23	0.00	60.13	0.00	58.50	0.00	49.93	0.39	32.65	0.42	34.63	0.67	31.83
	0.001	0.00	85.41	0.00	96.09	0.00	94.14	0.00	107.50	0.00	88.89	0.00	99.20	0.00	106.09
Majamil	1.000	1.39	11.75	4.13	7.11	10.70	5.25	10.14	5.57	14.46	1.56	11.56	0.00	20.22	0.00
	0.500	1.54	27.43	2.08	7.42	5.98	6.24	6.69	6.13	9.45	4.10	8.82	3.17	13.55	1.75
	0.100	0.57	30.96	0.71	17.70	1.84	10.66	2.49	10.96	3.68	9.63	3.52	7.08	6.54	6.70
	0.050	0.00	32.95	0.43	24.85	1.33	16.52	1.43	15.63	2.44	11.86	2.18	11.27	5.69	12.15
	0.020	0.00	33.19	0.00	26.93	0.00	24.44	0.00	24.36	1.01	21.57	0.83	20.12	2.40	18.15
	0.010	0.00	28.97	0.00	49.34	0.00	37.39	0.00	29.44	0.00	34.77	0.00	27.63	0.00	31.15
	0.001	0.00	81.16	0.00	76.96	0.00	78.20	0.00	69.72	0.00	74.97	0.00	81.73	0.00	79.32

i.A, % (w/w) concentration of impurity A with reference to active substance (diclofenac); **D**, diclofenac concentration (%, w/w).

Table 4
The results of diclofenac and 1-(2,6-dichlorophenyl)indolin-2-one (impurity A) determination for various concentrations of HCl at temperature 60 °C

Preparation	c (HCl) (mol/dm^3)	0 day		1 day		2 days		3 days		4 days	
		i.A	**D**	i.A	**D**	i.A	**D**	i.A	**D**	i.A	**D**
Olfen	1.000	0.00	34.70	34.41	1.13	51.51	0.00	52.74	0.00	35.60	0.00
	0.500	0.00	42.75	21.57	4.04	47.71	0.00	53.11	0.00	26.91	0.00
	0.100	0.00	41.81	16.69	26.27	42.06	20.12	50.60	10.43	31.72	4.37
	0.050	0.00	42.25	14.59	29.28	30.41	23.94	41.98	23.54	28.87	18.00
	0.020	0.00	69.92	7.31	40.49	17.17	27.45	22.82	26.62	18.51	25.90
	0.010	0.00	82.68	1.72	61.04	3.91	43.11	5.81	39.70	6.14	39.47
	0.001	0.00	90.61	0.22	89.79	0.56	89.58	0.89	84.89	0.45	73.41
	0.0001	0.00	101.74	0.57	100.55	1.93	96.39	2.47	71.67	3.37	66.96
	0.00001	0.00	103.96	0.79	97.83	1.79	76.69	2.45	69.89	3.00	64.18
	0.0000001	0.00	104.46	0.81	101.28	1.54	77.28	2.35	70.18	3.03	65.29
Dicloratio retard	1.000	0.00	21.07	10.24	0.00	13.23	0.00	13.53	0.00	13.56	0.00
	0.500	0.00	23.48	8.26	3.52	10.40	2.91	11.61	1.23	11.54	0.00
	0.100	0.00	26.01	2.89	7.95	8.04	4.41	11.48	3.27	11.60	0.00
	0.050	0.00	33.86	1.98	9.22	7.85	9.29	10.86	4.83	10.41	0.00
	0.020	0.00	43.80	1.55	31.33	6.67	22.89	8.38	19.22	12.17	7.68
	0.010	0.00	61.23	0.67	37.21	1.13	33.88	2.86	33.76	3.97	22.29
	0.001	0.00	85.41	0.00	104.83	0.67	100.03	1.71	107.66	2.94	104.03
	0.0001	0.00	103.31	0.00	105.77	1.92	97.13	2.07	97.14	2.16	96.68
	0.00001	0.00	107.41	0.00	110.28	1.54	107.78	1.45	105.98	2.10	97.71
	0.0000001	0.00	110.97	0.00	100.30	1.12	94.48	1.94	94.89	2.05	93.92
Majamil	1.000	1.39	11.75	17.52	0.00	30.88	0.00	19.76	0.00	21.59	0.00
	0.500	1.54	27.43	19.78	3.67	27.46	0.00	17.57	0.00	20.91	0.00
	0.100	0.57	30.96	28.74	9.26	21.91	6.61	11.89	7.03	23.13	1.55
	0.050	0.00	32.95	15.91	15.15	12.73	12.45	14.88	6.46	23.92	6.51
	0.020	0.00	33.19	9.71	29.59	2.78	21.02	5.13	17.05	12.32	11.88
	0.010	0.00	28.97	3.24	50.49	2.35	36.73	1.08	32.76	3.65	33.46
	0.001	0.00	81.16	0.00	101.26	0.35	91.96	1.07	87.98	3.35	89.20
	0.0001	0.00	98.30	0.00	101.45	2.82	93.17	2.44	93.13	5.08	91.44
	0.00001	0.00	99.44	0.00	100.34	1.24	96.81	1.55	93.35	2.67	90.85
	0.0000001	0.00	102.47	0.00	94.26	0.98	91.77	1.50	89.61	1.29	86.84

i.A, % (w/w) concentration of impurity A with reference to active substance (diclofenac); **D**, diclofenacu concentration (%, w/w).

3.5. Accuracy

The accuracy of the method was defined as% recovery of analyte added from 80 to 120% of relevant substances with respect to preparations examined. The following values of recovery were found for individual constituents (for $n = 3$), diclofenac: $\times$, 99.20%; relative standard deviation (R.S.D.), 0.61% $\mu_{95\%}$, $99.20\% \pm 1.51$; impurity A: $\times$, 92.34%; R.S.D., 1.35% $\mu_{95\%}$, $92.34\% \pm 3.10$; impurity E: $\times$, 95.85%; R.S.D., 4.18% $\mu_{95\%}$, $95.85\% \pm 9.98$.

The results obtained were used for developing the method of quantitation for further investigations.

3.6. Precision

The precision of the method was expressed as a consistence degree between the results of analyses carried out repeatedly. The tests were conducted for a model mixture solution containing diclofenac (0.025%), impurity A (0.023%) and impurity E (0.055%). To determine the results consistency measurements of peak areas for each constituent were done. The scatter of results was characterised by the standard deviation (S.D.) which also confirms reproducibility of the method and the R.S.D. in compliance with the arithmetic mean ($\times$) from six measurements. For each constituent the following results were obtained, diclofenac: from 3492.0 to 3780.1, $\times$, 3630.6; *S*, 108.5; R.S.D., 2.99%; impurity A: from 2718.8 to 2810.3, $\times$, 2756.7; *S*, 32.6; R.S.D., 1.18%; impurity E: from 1481.6 to 1587.2, $\times$, 1523.3; *S*, 39.9; R.S.D., 2.62%.

3.7. Quantitative analysis

Standard solutions, 4 μl of diclofenac and impurity A, 2 μl of impurity E and preparation solutions of 1 μl for determination of diclofenac and of 20 μl for impurities were applied in the form of 1 cm bands on chromatographic plates of 9×12 cm. Chromatograms were developed over the mobile phase distance of 12 cm by using the mobile phase, cyclohexane–chloroform–methanol (12:6:1, v/v/v), and then dried at room temperature. The densitograms were recorded at the wavelength of $\lambda = 248$ nm.

The concentrations of each constituent in preparations examined were determined by comparing the peak areas for appropriate standard and sample solutions.

The results of diclofenac determination are presented in Table 1. The results for impurities have not been included, as no impurities were found.

To confirm the lack of impurities, HPLC was used as a comparative method [2].

3.8. Determination of impurity A by HPLC

Standard solutions and corresponding preparation solutions of 20 μl each were applied sequentially on the column of 250×4 mm, packed with octadecylosilane chemically bound on the surface of amorphous silica (Lichrosorb RP-18) and the flow rate of mobile phase was fixed to 1 ml per min. The detection was made in UV at $\lambda = 254$ nm.

Individual peaks on chromatograms were identified by comparing the retention times for standard solutions and for the solutions examined. The peak areas were used for determining impurity A.

Examples of chromatograms for standard solution of impurity A and preparation solution are shown in Fig. 4.

Similar chromatograms were obtained for all preparations and no peaks at the retention time for impurity A (ca. 7.7 min) were noted. The only single strong peak at the retention time for diclofenac (ca. 12.3 min) was visible on all chromatograms.

The results have confirmed the reliability of data obtained by the chromatographic-densitometric method which also revealed no impurities.

3.9. The effect of pH and temperature on concentrations of diclofenac and impurities in model solutions of selected drugs

The examination was carried out on the following drugs: Dicloratio retard 100 (capsules), Olfen 75 (injections) and Majamil 50 (tablets). The solutions for examination were prepared from pow-

dered tablets by weighing 20 mg of diclofenac or taking an appropriate volume of injections. After dissolving in 20 ml of hydrochloric acid at concentrations ranging from 1 to 10^{-7} mol/l (pH from 0 to 7), the samples were diluted with methanol (in 1:1, v/v) at time intervals specified in Tables 2–4. The chromatographic-densitometric method was employed. The chromatogram spots were identified based on the absorption spectra and R_f values.

The areas of appropriate peaks were used for quantitation. The results are presented in Tables 2–4.

A decreasing concentration of impurity A was found after incubation of diclofenac solutions at 60 °C on day 4. This can indicate, for example, decomposition of that impurity. Additional examinations were carried out and this hypothesis was confirmed by the appearance of an additional peak at $R_f \approx 0.95$.

3.10. The effect of UV on concentrations of diclofenac and impurities in model solutions

The drugs mentioned above were examined. Sample solutions were prepared from powdered tablets by weighing an amount corresponding to 20 mg of diclofenac or taking an appropriate volume of injection. After dissolving in 20 ml of methanol–water (1:1, v/v) and filtering, the samples were exposed to UV at $\lambda = 254$ and 366 nm at time intervals specified in Table 5. The chromatogram spots were identified by densitometric measurements. The results are presented in Fig. 6.

The chromatograms of samples indicated the presence of diclofenac and two additional spots originated from substances of R_f values different from those of the impurities (A and E) examined. Thus, it was concluded that after UV irradiation, new compounds denoted as **Y** and **Z**, appeared. These compounds have not been analysed in detail due to the lack of appropriate standard substances. The quantitation was confined only to determination of concentrations for diclofenac and other compounds by using the internal normalisation, from the following formula, $\% i = x_i / \Sigma x$, where, $\% i$, constituent concentration i [%]; x_i, peak area for constituent under examination; Σx, sum of peak areas. The results are shown in Table 5.

4. Discussion

A new method that combines TLC and densitometry was developed for simultaneous identification and quantitation of the active substance and its impurities in order to assess the quality of diclofenac and impurities 1-(2,6-dichlorophenyl)-indolin-2-one (impurity A) and indolin-2-one (impurity E) and fulfilling the requirements specified in the European and British Pharmacopoeias. In this study two of five impurities listed in the pharmacopoeias were analysed [2,3].

The conditions for separation of diclofenac and analysed impurities and the method for chromatographic identification were established by using appropriate standard solutions.

It was found experimentally that the mobile phase, cyclohexane–chloroform–methanol (12:6:1, v/v/v) provided good constituent separation, and at the same time gave well developed peaks on densitograms what is of utmost importance in densitometric analysis. The constituents differ not only in their position on the chromatograms (diclofenac $R_f \approx 0.32$, impurity A $R_f \approx 0.67$, impurity E $R_f \approx 0.21$) but also in the absorption spectra in terms of specific absorbance maximum and shape. These parameters appear to be of a great significance in quantitation and identification (Fig. 2).

The method presented above meets all general requirements of good laboratory practice in terms of specificity, with respect to potential matrix constituents, originated from vehiculum or other active substances in complex medicines. Under established conditions selective determination of diclofenac, as well as impurities appearing together and in the presence of other constituents such as misoprostol ($R_f \approx 0.00$) and lidokaine ($R_f \approx 0.13$) was possible. No influence of other substances originated from the matrix in the studied pharmaceutical preparations obtained from different manufactures was observed. The method is characterised by good precision, very high sensitivity and constituent detection (diclofenac 0.13

Table 5
The results of determination for diclofenac and products of its decomposition under UV irradiation (concentrations calculated by the internal normalisation method described in the paper)

Preparation	λ (nm)	Determination product	0	15 min	30 min	45 min	60 min	75 min	90 min	105 min	120 min
Olfen	254	Diclofenac	96.62	93.31	82.87	80.45	80.44	76.19	74.79	70.08	68.30
		Y	3.38	2.99	6.91	7.96	8.41	10.55	12.17	14.13	15.28
		Z	0.00	3.69	10.23	11.83	11.14	13.26	13.03	15.79	16.42
	366	Diclofenac	96.62	91.47	88.82	86.32	84.63	82.41	81.77	79.06	75.60
		Y	3.38	5.95	6.85	8.39	8.42	8.78	10.99	12.16	13.81
		Z	0.00	2.57	4.33	5.29	6.95	8.81	7.25	8.78	10.59
Dicloratio retard											
	254	Diclofenac	100	95.28	90.16	87.19	82.64	82.26	79.14	75.65	73.07
		Y	0.00	0.71	2.57	3.90	6.88	9.57	8.74	11.02	12.40
		Z	0.00	3.86	7.27	8.91	10.48	9.57	12.12	13.32	14.53
	366	Diclofenac	100	98.09	93.74	91.16	89.51	87.24	87.92	85.06	83.78
		Y	0.00	0.00	1.91	2.75	3.95	4.75	5.71	6.45	7.88
		Z	0.00	1.91	4.35	6.08	6.53	8.01	6.38	8.49	8.34
Majamil	254	Diclofenac	100	95.09	94.12	92.77	92.36	90.45	87.73	86.52	70.40
		Y	0.00	2.04	2.30	2.54	2.80	4.16	5.52	5.85	13.94
		Z	0.00	2.87	3.57	4.69	4.84	5.40	6.75	7.63	15.67
	366	Diclofenac	100	93.50	90.22	88.40	85.99	82.91	80.20	78.20	74.63
		Y	0.00	1.43	2.84	3.53	4.35	7.25	9.05	10.02	12.50
		Z	0.00	5.07	6.94	8.06	9.66	9.84	10.75	11.78	12.87

μg, impurity A 0.20 μg, impurity E 0.05 μg), wide range of linearity (0.007–0.072%, w/v for diclofenac; 0.005–0.050%, w/v for impurity A; 0.005–0.055%, w/v for impurity E) and high recovery (99.20%, w/v for diclofenac; 92.34%, w/v for impurity A; 95.85%, w/v for impurity E).

It is important to point out that UV measurements at the analytical wavelength $\lambda = 248$ nm common to all constituents gave correct and repeatable results.

The examinations carried out on selected preparations were confined only to the determination of diclofenac, as no impurities were found. The results presented in Table 1 are characterised by high accuracy, repeatability and precision, as confirmed by statistical analysis. For diclofenac determination, the confidence intervals for each preparation ranging from 1.69 to 4.33% and R.S.D. from 1.81 to 4.75% were obtained. Some doubts as to the suitability of the method could arise mainly from the negative results obtained for impurity A, the level of which has been specified in the European Pharmacopoeia. The results obtained for the model mixture based on peak areas measurements in quantitative analysis confirm good precision of the method. The calculated values of S.D. and R.S.D. are comparable, what suggests that the results obtained under the established conditions of determination for each constituent are of a comparable quality. Thus, an additional determination of impurity A was carried out by HPLC, as recommended in the pharmacopoeia. The reliability of the densitometric method has been confirmed, as the obtained chromatograms showed only peaks at the retention time typical of diclofenac (ca. 12.3 min). For all preparations examined no peaks at the retention time typical of impurity A (ca. 7.7 min) were found.

In further investigations, based on the published reports [5], the effect of pH in aqueous solutions on diclofenac decomposition was

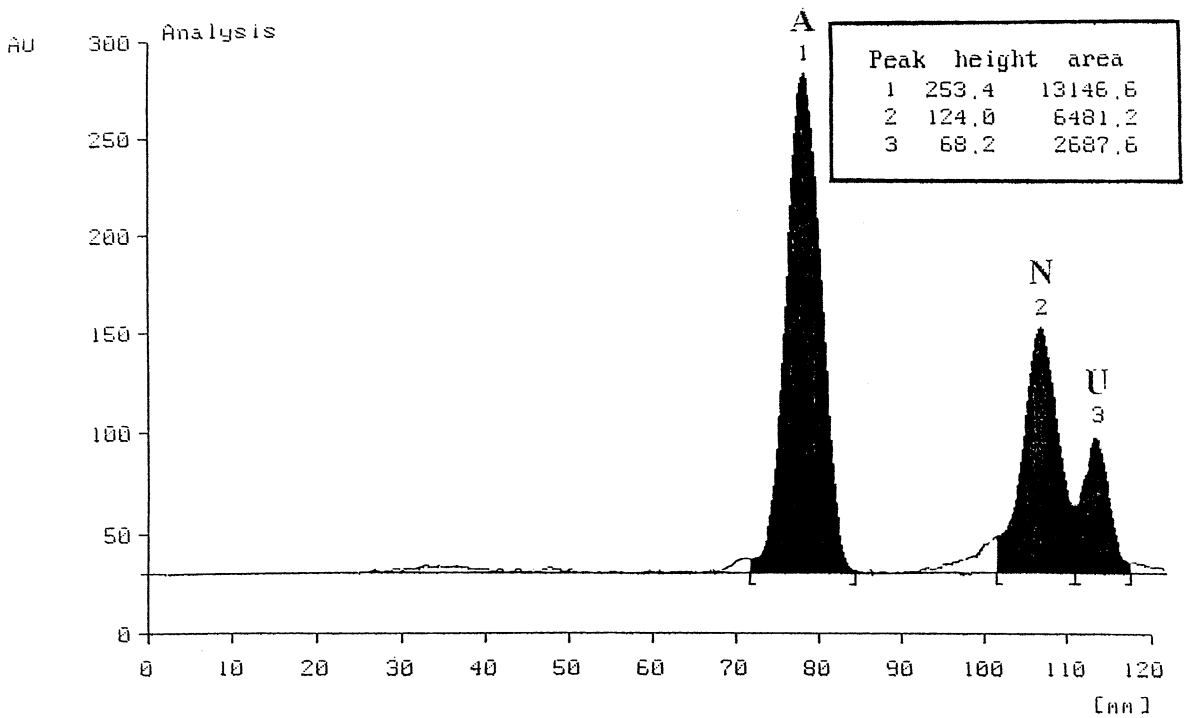
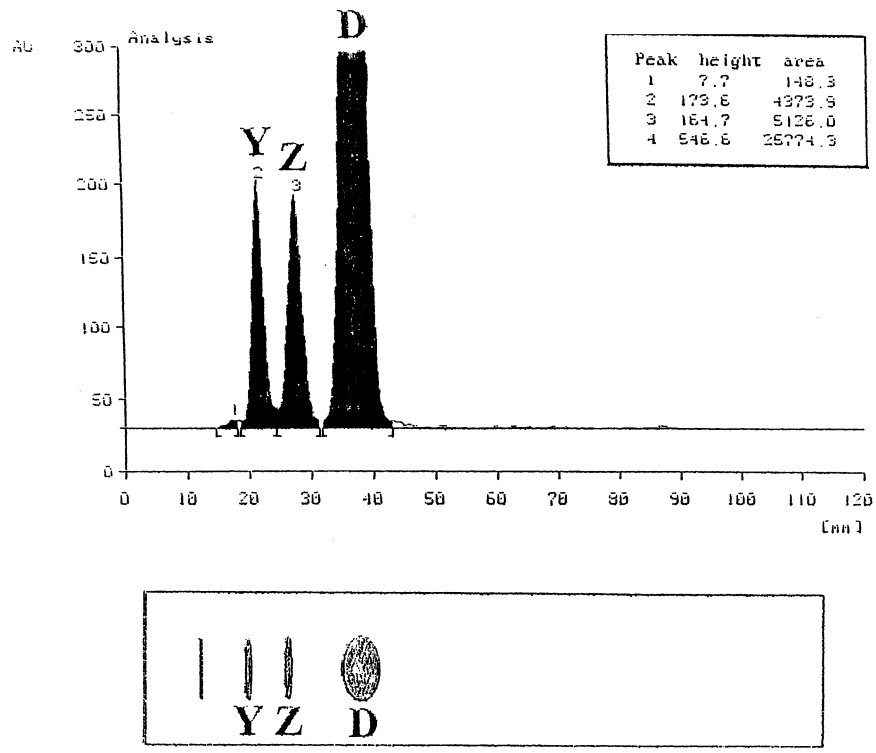


Fig. 5. An example densitogram for impurity A (A) and unidentified product (N) arisen after 4-day incubation at 60 °C. The peak marked with U is the front of a mobile phase.

a) densitogram and chromatogram



b) absorption spectra

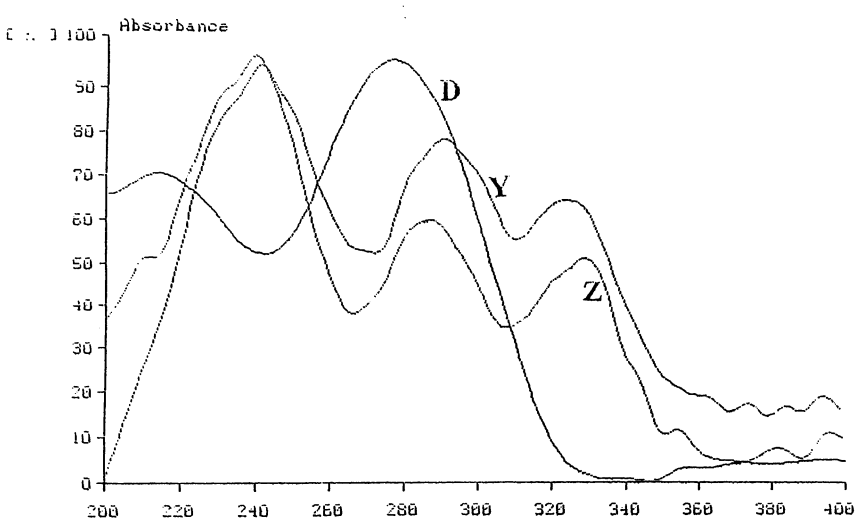


Fig. 6. An example of densitogram and chromatogram of diclofenac (**D**) and two unidentified compounds (**Y** and **Z**) obtained after UV sample irradiation (a), and absorption spectra of both products recorded directly from chromatogram (b).

analysed. When determining diclofenac and impurity A (as no traces of impurity E were detected) it has been found that the decomposition of diclofenac is faster in solutions with lower pH and depends on the temperature.

The fastest decomposition of diclofenac was observed in solutions of high acidity. The higher pH, the slower decomposition of diclofenac. An increasing temperature considerably speeds up the decomposition process. A temperature rise up to 60 °C led to a complete decomposition of diclofenac within 24 h (Tables 2–4).

The changes in diclofenac concentration were accompanied by a proportional increase in the concentration of impurity A, depending on pH and temperature of the solution. After 4-day incubation at 60 °C (Table 4) some changes resulting probably from decomposition of impurity A were observed. The concentration of impurity A decreased in comparison to that of three previous days (Fig. 5).

In addition, the effect of UV on the stability of diclofenac was analysed (Fig. 6). Densitograms showed both the peaks generated by diclofenac ($R_f \approx 0.32$), as well as unidentified substances denoted as **Y** ($R_f \approx 0.19$) and **Z** ($R_f \approx 0.23$). As the product of diclofenac decomposition remains unknown, the internal normalisation was used for quantitation to determine both diclofenac and the products of its decomposition.

The examinations carried out at $\lambda = 254$ and 366 nm have indicated no significant differences in the decomposition process. In both cases similar phenomena were observed. The results listed in Table 5 show decreasing concentration of diclofenac after UV irradiation, thus indicating gradual decomposition induced by UV. The changes in diclofenac concentration were accompanied by corresponding changes in concentrations of **Y** and **Z**. These products of diclofenac decomposition differ from impurities A and E both in peak positions and in the absorption spectra (Fig. 5).

5. Conclusions

A chromatographic-densitometric method for simultaneous determination of diclofenac and 1-(2,6-dichlorophenyl)indolin-2-one and indolin-2-one was developed. Based on the results presented above it has been demonstrated that the method can be used as an alternative to HPLC, both in diclofenac purity assessment, as well as in kinetic analysis.

The developed method is characterised by high sensitivity, selectivity and accuracy. This method is worth recommending because of a short time of analysis, little usage of reagents and simplicity.

It has been also shown, that diclofenac decomposes in aqueous solutions releasing 1-(2,6-dichlorophenyl)indolin-2-one (impurity A), the concentration of which depends on pH and temperature of the solution.

An adverse effect of UV on the stability of diclofenac in model solutions was found. UV irradiation reduced the concentration of diclofenac, while generating new products of different physical and chemical properties.

This finding appears to be interesting for technology not only in terms of drug formulation but also in therapy, especially in the case of drugs administered orally as they are exposed to the acid environment of the stomach.

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