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LC method for the quantitative determination of oxaprozin and its impurities in the bulk drug☆

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Abstract

A reversed phase linear gradient liquid chromatographic method was developed for the separation and quantitative determination of the seven known process related impurities and one degraded product of oxaprozin in the bulk drug material. An Inertsil-ODS 3V (150 × 4.6 mm), 5 μm column was operated with a phosphate buffer–acetonitrile gradient. Detection was carried out on a UV detector at 254 nm. This method has been proved to be accurate and sensitive. The limits of detection (LOD) and limits of quantification (LOQ) of impurities were in the order of 5–60 ng and 16–200 ng, respectively. In addition to its ruggedness and robustness, this method offers identification of all eight impurities in a single run.

Keywords: Oxaprozin; Impurities; Gradient liquid chromatography; LOD; LOQ

1. Introduction

Oxaprozin (Fig. 1), chemically known as 4,5-diphenyl-2-oxazole propionic acid [1], is best known as a non-steroidal anti-inflammatory agent which is used for the treatment of pain, inflammation and rheumatic conditions [2,3]. Several chromatographic methods have been described in literature for the determination of oxaprozin. It was separated and identified both by thin layer chromatography [4,5] and gas chromatography [6]. A number of liquid chromatographic (LC) methods were reported for the determination of oxaprozin [7–10] in biological fluids. However, these methods can not be used for the determination of the bulk drug in the presence of starting materials and by-products. An LC method is described for the quantitative determination of oxaprozin [11] and several of its related impurities. This method involved the use of a rather complex mobile phase containing 1-decanesulfonic acid as ion-pairing agent. The flow rate was increased for the elution of methyl ester of oxaprozin, which still eluted after 42 min. The same paper also described a solvent system for the elution of tetraphenylpyrazine, a by-product during the synthesis of oxaprozin. The present paper describes a new, simple more and reliable LC method in gradient mode. This method is not only

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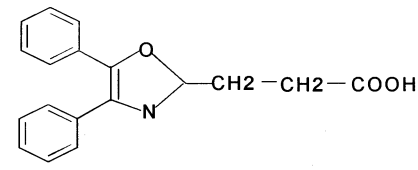

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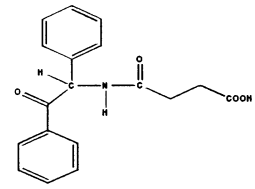

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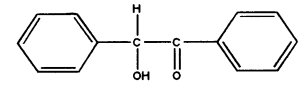

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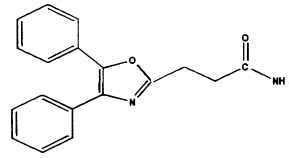

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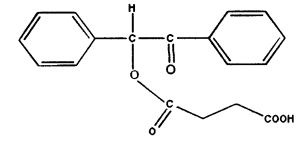

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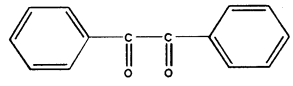

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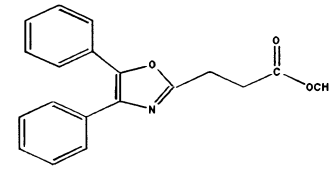

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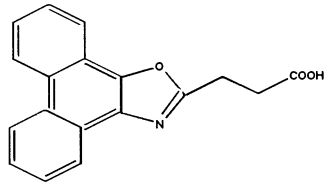

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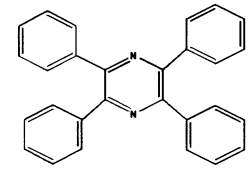

(h)

Fig. 1. Chemical structure of oxaprozin and its impurities. I, oxaprozin; (a), N-[(α-phenyl) phenacyl]succinamic acid; (b), benzoin; (c), 4,5-diphenyl-2-oxazole propionamide; (d), benzoin hemisuccinate; (e), benzil; (f), methyl-4,5-diphenyl-oxazole-2-propionate; (g), phenanthro[9,10]-oxazole-2-propionic acid; (h), tetra phenyl pyrazine.

useful for the detection of all the process-related impurities, degradation products and byproducts but also for the quantitative determination of oxaprozin in the presence of the impurities which may be present in the bulk drug. High–Low chromatographic technique [12] was applied for the quantification of impurities.

2. Experimental

2.1. Instrumentation

An LC system consisting of a Perkin Elmer low pressure gradient solvent delivery module (Model No. PE-250), Photo-diode array detector (Model Waters PDA 996) and a manual injector (Model 7725i Rheodyne) was used. Chromatographic data were monitored by using the Waters Millennium 2010 Chromatography manager software. The column temperature was maintained at constant temperature of 27°C using a Waters column oven. pH adjustment of aqueous phase (solvent A) composition was done using Elico pH meter (Model No. L120) pH meter.

2.2. Reagents and chemicals

HPLC grade acetonitrile and methanol were obtained from E.Merck (Germany) and HPLC grade water was collected through a Millipore Milli-Q water purification system. AR grade potassium dihydrogen phosphate was purchased from SD-Fine Chem (India).

AR grade ortho phosphoric acid came from Qualigens (Glaxo India Ltd.) and was used for making necessary pH adjustments.

Oxaprozin (pharma grade) and its impurities were supplied by one of our process R&D laboratories.

Oxaprozin (1) and all the other impurities namely N-[(α-phenyl) phenyl] succinamic acid (a), benzoin (b), 4,5-diphenyl-2-oxazole propionamide (c), Benzoin hemisuccinate (d), benzil (e), methyl-4,5-diphenyl-oxazole-2-propionate (f) (all process related) and phenanthro [9,10]-oxazole-2-propionic acid (g) (degradation product) and tetra phenyl pyrazine (h) (byproduct) were supplied by Process R&D laboratory of Dr Reddy's Research Foundation. Chemical structures of oxaprozin and impurities are shown in Fig. 1.

2.3. Preparation of mobile phase

2.3.1. Preparation of buffer solution

2.3.1.1. Phosphate buffer (0.01M). Buffer solution was prepared by dissolving 0.96 g of potassium dihydrogen phosphate in 700 ml of Milli-Q water.

2.3.2. Preparation of solvent A

Solvent A was prepared by mixing 70 parts (v/v) of buffer solution and 30 parts (v/v) of acetonitrile. The resulting mixture was shaken and the pH was adjusted to 3.5 with 75% (v/v) H_3PO_4. The mixture was filtered through a 0.45 μm nylon 66 membrane using a Millipore vacuum filtration system.

2.3.3. Preparation of solvent B

Filtered and degassed acetonitrile (HPLC grade) was used as solvent B.

2.4. Preparation of sample solutions

2.4.1. Preparation of oxaprozin standard solution

About 50 mg of oxaprozin was weighed into a 10 ml volumetric flask. The sample was first dissolved in 3–4 ml of methanol under sonication and then made up to volume with acetonitrile. This solution was labeled as standard stock solution.

2.4.2. Preparation of oxaprozin impurity solutions

About 5.0 mg of each impurity was separately weighed into a 5.0 ml volumetric flask. Each sample was dissolved in 1.5–2 ml of acetonitrile. Impurity 'h' was dissolved in 0.5 ml of toluene and was made up to volume with acetonitrile.

For checking the linearity, both the oxaprozin standard stock solution as well as the impurity solutions were prepared in the range of 20–150% and 0.1–2.0% respectively of the target analyte concentration (0.5 mg/ml).

2.5. Analytical LC conditions

An Inertsil-ODS 3V (150 × 4.6 mm, 5.0 μm) column (GL Sciences make) was used for analysis of the samples. Gradient analysis was performed with a linear gradient using the following program. Solvent A: potassium dihydrogen ortho phosphate (10 mM)–acetonitrile (70:30, v/v) (pH 3.5). Solvent B: Acetonitrile.

The gradient programme is given in Table 1.

The flow rate was set at 1.0 ml/min and the detection was carried out at a wavelength of 254 nm.

3. Results and discussions

3.1. Method development and validation

Ibrahim [12] described the determination of oxaprozin and several of its impurities with isocratic LC using a complex mobile phase containing 1-decanesulphonic acid as an ion-pairing agent. Furthermore, the flow rate was increased during the analysis in order to elute the methyl ester of oxaprozin and a different mobile phase was used for the elution of another impurity tetraphenylpyrazine, which is a by-product of the synthesis of oxaprozin. To avoid the use of ion-pairing agents, flow rate variations and different mobile phases a gradient LC method was developed enabling the separation and quantitation of all the possible impurities in oxaprozin in a single run. For this purpose several methods were screened involving different mobile phases by using several ODS LC columns. Almost all the columns, except the Inertsil-ODS 3V (150 × 4.6 mm), 5.0 μm, yielded either coelution of some of the peaks or unacceptable band broadening. An Inertsil-ODS 3V (250 × 4.6 mm), 5.0 μm was also tested, but this resulted in longer retention times and total run time. As a result, an accurate and reliable LC method was developed for the determination of oxaprozin and its impurities using the 150 mm column. Using this column all compounds were adequately separated. A representative LC chromatogram of oxaprozin and its impurities is shown in Fig. 2. The resolution factor (Rs) between any pair of peaks was found to be over 2.

Table 1
LC conditions used for the analysis of oxaprozin and its impurities

Time (min)	Solvent A (%)	Solvent B (%)
0	96	4
20	96	4
30	40	60
40	0	100
50	0	100
60	96	4

Samples (10 μl) of oxaprozin in volumes were injected at different concentrations into LC and the response of the oxaprozin peaks was recorded. Solutions (20 μl) of the impurities were injected at different concentrations into LC and the response of the impurities was recorded. Calibration plots were made both for oxaprozin and the impurities. Oxaprozin exhibited good linearity in the concentration range of 20–150% of target analyte concentration. The impurities exhibited good linearity in the concentration range of 0.1–2.0% of target analyte concentration. The relative retention times (RRT), relative response factors (RRF) of oxaprozin and all the impurities along with their correlation coefficients are shown in Table 2. The equation for the calibration curve is $y = ax + b$. The RSD values for three determinations for the slope and the intercept for the calibration curves of oxaprozin and its impurities are given in Table 2.

3.2. Recovery studies of impurities

Oxaprozin at a concentration of approximately 1.0 mg/ml was spiked with impurity solutions of concentrations of 0.5, 1.0 and 1.5%. A volume of 20 μl was loaded onto the LC column and the responses of the impurities were recorded. The recoveries are presented in Table 3. The recoveries ranged from 95 to 107%.

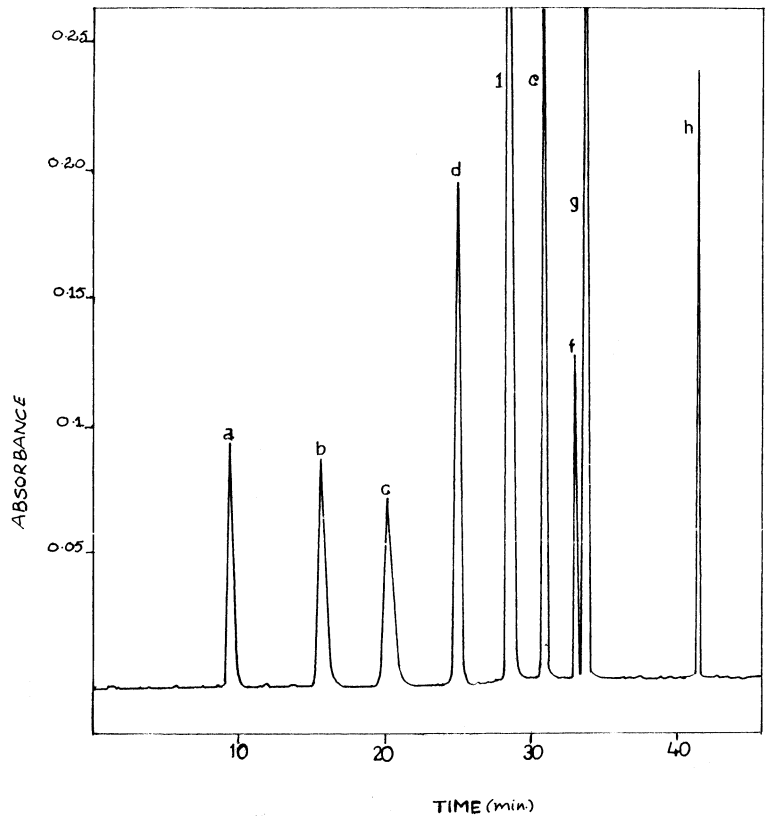

Fig. 2. HPLC chromatogram of oxaprozin and its impurities (for LC conditions see text).

Table 2
Relative retention times (RRT), relative response factors (RRF), correlation coefficients (r^2) and relative standard deviation (RSD) values of slopes and intercepts of oxaprozin and its related substances

Compound	RRT	RRF	(r^2)	$\bar{X}^a$	RSD[b]	$\bar{X}^c$	RSD[d]
Oxaprozin	1.00	1.00	0.999	144576.9	6.35	0.00054	6.93
a	0.33	0.76	0.998	112054.2	5.34	19636.9	9.37
b	0.55	2.01	0.995	316797.3	8.16	49496.4	4.08
c	0.72	1.03	0.992	196949.4	8.38	30271.6	6.09
d	0.88	1.40	0.997	228965.1	9.67	17381.2	11.34
e	1.09	3.66	0.996	611109.2	2.53	10814.3	7.22
f	1.17	0.78	0.986	129012.9	9.96	81114.3	3.15
g	1.19	3.69	0.989	113744.9	2.30	18208.7	9.97
h	1.46	2.88	0.991	349092.3	3.20	53090.3	3.98

[a] Mean value of slope ($n = 3$).
[b] RSD values of slope.
[c] Mean value of intercept ($n = 3$).
[d] RSD values of intercept.

3.3. Ruggedness

The method ruggedness of oxaprozin impurities was studied by using a different instrument, a Waters LC consisting of a model 510 low pressure gradient solvent delivery unit, a model 486 tunable UV detector and a manual Rheodyne injection valve. The experiments were performed in a different laboratory, using different lots of reagents but at the same concentration level. The results thus obtained were about the same as found in a repeatability study and the RSD was less than 2%. Data obtained for the same concentration levels from laboratory A and laboratory B for one day repeatability study are given in Table 4.

3.4. Robustness

The robustness of the method was tested in terms of variation in pH (3.0 and 4.0), solvent strength ($\pm$ 2.0% of acetonitrile in solvent A) and flow rate (0.8 and 1.2 ml/min). It was observed that at pH 3.0 the retention times of oxaprozin and its impurities were slightly longer and vice versa in case of pH 4.0. When the solvent strength was increased by 2% in solvent A, retention times only slightly decreased. The retention times were found to decrease with increase in flow rate.

However, in either of these conditions there has been no significant change in the results obtained. The results of robustness study are presented in Tables 5–7.

Table 3
Recovery studies of oxaprozin impurities

Impurity	Added (μg/ml) ($n = 3$)	Found (%)	Recovery (%)	RSD (%)
a	5.3	5.9	109.25	0.74
	10.2	11.0	107.84	
	15.2	16.4	107.89	
b	5.2	5.1	98.07	0.05
	10.6	10.4	98.11	
	15.1	14.8	98.01	
c	4.8	4.9	102.08	0.40
	11.5	11.7	101.73	
	15.8	16.2	102.53	
d	5.2	5.1	98.07	0.08
	10.9	10.7	98.16	
	15.1	14.8	98.01	
e	4.9	4.8	97.95	0.36
	11.1	10.8	97.29	
	15.5	15.1	97.41	
f	5.1	5.3	103.92	0.20
	10.9	11.3	103.62	
	15.6	16.2	103.84	
g	4.9	4.8	97.95	0.30
	11.5	11.2	97.39	
	16.1	15.7	97.51	
h	4.9	5.2	106.12	0.20
	10.7	11.4	106.54	
	15.8	16.8	106.32	

Table 4
Repeatability studies of oxaprozin impurities performed in laboratory A and B

Impurity	Concentration added (μg/ml) (lab A & B)	Recovered (%) ($n = 3$) (lab A)	RSD (%) (lab A)	Recovered (%) ($n = 3$) (lab B)	RSD (%) (lab B)
a	5.29	106.23	0.80	107.75	0.71
	10.15	108.47	0.79	107.88	1.25
	15.31	105.87	0.64	105.36	0.82
b	5.32	98.68	0.68	97.93	0.99
	10.52	98.28	1.12	99.62	0.85
	15.30	99.02	1.24	99.41	1.04
c	5.15	101.55	0.85	101.94	0.70
	10.81	101.94	1.01	101.57	0.82
	14.92	101.34	0.96	100.67	1.12
d	5.42	98.71	0.82	101.85	0.67
	10.75	98.23	1.09	98.69	0.78
	15.29	98.88	0.69	99.67	0.88
e	5.05	98.02	1.37	101.58	1.11
	11.29	98.75	1.33	99.29	1.16
	15.60	98.20	1.47	99.04	1.02
f	4.82	102.69	0.98	103.31	0.98
	10.79	101.11	1.11	100.55	1.26
	15.72	100.83	1.02	101.14	1.13
g	4.75	102.10	1.06	103.57	1.28
	11.29	101.06	1.76	100.53	0.91
	16.25	101.85	1.44	101.05	1.13
h	5.19	102.50	1.02	101.92	1.26
	10.52	101.61	1.32	100.57	0.83
	15.62	101.28	1.38	100.64	1.28

Table 5
Effect of change in pH on the assay results of oxaprozin and its impurities

Compound	Concentration taken (μg/ml)	Recovery (%)			RSD (%)
		Normal conditions	Altered conditions		
		pH 3.5	pH 3.0	pH 4.0	
I	533.4	99.94	101.19	100.14	0.67
a	10.52	99.61	102.28	102.75	1.66
b	10.91	96.42	94.32	98.62	2.23
c	10.78	96.38	98.14	96.47	1.02
d	9.92	98.89	103.32	99.69	2.35
e	11.25	97.96	96.80	96.44	0.82
f	11.06	102.53	101.71	99.27	1.68
g	10.83	97.32	101.75	98.71	2.28
h	11.75	96.68	98.89	101.53	2.45

Table 6
Effect of change in flow rate on the assay results of oxaprozin and its impurities

Compound	Concentration taken (μg/ml)	Recovery (%)			RSD (%)
		Normal flow rate (ml/min)	Altered flow rate (ml/min)		
		1.0	0.8	1.2	
I	533.4	100.52	101.25	100.37	0.46
a	10.52	99.04	103.23	98.09	2.73
b	10.91	97.43	101.01	96.79	2.31
c	10.78	97.22	101.29	96.38	2.67
d	9.92	98.69	103.32	97.28	3.17
e	11.25	97.96	97.33	94.49	1.91
f	11.06	100.72	102.44	98.37	2.03
g	10.83	97.05	101.66	96.49	2.88
h	11.75	98.38	100.59	95.57	2.56

Table 7
Effect of change in solvent content (solvent A) on the assay results of oxaprozin and its impurities

Compound	Concentration taken (μg/ml)	Recovery (%)			RSD (%)
		Normal conditions	Altered conditions		
		30%[a]	28%[a]	32%[a]	
I	533.4	100.21	100.71	100.51	0.25
a	10.52	99.71	102.47	98.00	2.25
b	10.91	97.34	95.14	94.68	1.48
c	10.78	97.77	100.83	95.64	2.66
d	9.92	99.69	102.32	98.59	1.91
e	11.25	98.58	101.78	97.07	2.43
f	11.06	99.82	102.44	98.37	2.06
g	10.83	98.71	102.95	97.05	3.06
h	11.75	101.11	102.38	98.81	1.80

[a] % of acetonitrile in solvent A.

3.5. Limit of detection (LOD)

The limit of detection for all of the impurities ranged from 5.0 to 60 ng depending on the response of the compound. Table 8 displays the LOD values of impurities calculated with signal to noise ratio of three are given. An acetonitrile blank was injected several times to calculate signal to noise ratio.

3.6. Limit of quantification (LOQ)

The limit of quantification of impurities ranged from 16 to 200 ng depending on the response of the respective compound. The LOQ values, which are ten times the signal to noise ratio, are shown in Table 8. An acetonitrile blank was injected several times to calculate signal to noise ratio.

Table 8
Limit of detection (LOD) and limit of quantification (LOQ) for oxaprozin impurities

Compound	LOD[a] ($n = 3$) (ng/ml)	LOQ[a] ($n = 3$) (ng/ml)
a	60	200
b	15	50
c	32	106
d	25	83
e	08	26
f	31	33
g	05	16
h	12	40

[a] RSD is 4–12%.

3.7. Quantification of impurities

High-low chromatography [12] was applied for quantification of the impurities. Impure samples of oxaprozin, of different batches, were selected for this quantification study. Samples at a concentration of 10 mg/ml were injected into the LC and the chromatograms were recorded. The response factors of each impurity, with respect to oxaprozin, were established. The actual amount of the impurity in terms of weight percentage is calculated using the following equation.

$$\text{Wt\% of impurity} = \frac{\text{RFS} \times 100}{\text{RFST} \times \text{RRF}}$$

Where, RFS is the response factor of a given impurity in the sample, which is calculated by dividing the average area of the impurity in the sample by concentration of the sample.

RFST is the response factor of the oxaprozin standard, which is calculated by dividing the average area of the oxaprozin standard by its concentration. RRF is the relative response factor, which is calculated by dividing the response factor of a given impurity by the response factor of the standard.

4. Conclusions

The developed LC method can be applied for the simultaneous determination of Oxaprozin and related substances in a bulk drug in a single run. Quantification of major impurities of oxaprozin can be performed with this method. Because no extraction or other sample clean-up step involved, the proposed method is rapid and easy to perform. The sensitivity of the method is sufficient to monitor oxaprozin impurities in bulk products. The method was proven to be superior to the other reported methods in terms of short analysis time, detection and quantitation of all possible impurities in the bulk sample of oxaprozin.

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