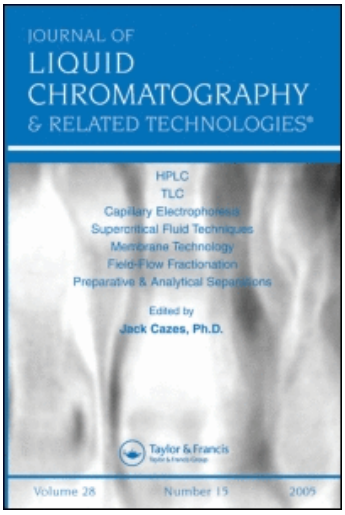


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# Quantitative Determination of Oxaprozin and Several of Its Related Compounds by High-Performance Reversed-Phase Liquid Chromatography

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# QUANTITATIVE DETERMINATION OF OXAPROZIN AND SEVERAL OF ITS RELATED COMPOUNDS BY HIGH-PERFORMANCE REVERSED-PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

An accurate, sensitive, and selective high-performance liquid chromatographic method for the quantitative determination and the separation of oxaprozin from several of its related compounds (including some precursors) has been developed. Reversed-phase ion-pair chromatography, with 1-decanesulfonic acid sodium salt as the ion pairing agent, was used. A $\mu$ Bondapak $C_{18}$ column with a mobile phase consisting of 0.01 M $KH_2PO_4$, methanol, and acetonitrile (2:1:1), pH 4.2, was used at a flow rate of 1 mL/min. Oxaprozin and its related compounds were monitored at 254nm. The method is rapid because no sample extraction is involved. Precision and ruggedness (relative standard deviation) on oxaprozin and several of its related compounds were below 1.0%. Detection limits ranged from 0.05 $\mu$g/mL to 0.08 $\mu$g/mL depending on the compound. The method is stability-indicating, and has applications in bulk pharmaceutical manufacturing.

## INTRODUCTION

Oxaprozin (4,5-diphenyl-2-oxazolepropionic acid) is a non-steroidal anti-inflammatory agent (1,2) used for the treatment of rheumatic and inflammatory conditions.

Several methods for the determination of oxaprozin are cited in the literature. It was separated and identified by thin-layer chromatography (3), determined by gas-liquid chromatography (4), and high-performance liquid chromatography (HPLC) (5-12).

Many of the above methods were used for determining oxaprozin in biological fluids, and are not suited to the problems encountered in bulk pharmaceutical manufacturing such as detecting unreacted starting materials, intermediates, and by-products, as well as completion reactions and kinetic studies.

The present report describes a rapid and sensitive technique to determine oxaprozin and several of its related compounds in pharmaceutical production, including wet cake, mother liquor and reaction completion samples.



## EXPERIMENTAL

### Apparatus

The system was a Varian 5000 liquid chromatograph with an 8055 autosampler (both by Varian Instrument Division, Walnut Creek, CA) and a 783A programmable absorbance detector by Applied Biosystems, Foster City,

CA. The diode array instrument was a Model 9010 pump, a Model 9095 autosampler and a Model 9065 photodiode array detector (all by Varian Instrument Division). System control was via the Series 9020 *LC* Star Workstation, Revision C (Varian Instrument Division). Spectral manipulation and comparison were performed using Polyview™ Revision D (Varian Instrument Division). The LC control and spectral processing software functions in a Windows™ environment (Windows Version 3.0, Microsoft Corporation, Redmond, WA) on a 386 PC equipped with a math coprocessor. A 300 x 3.9 mm $\mu$ Bondapak $C_{18}$ column (Waters Division of Millipore, Milford, MA) was used.



Reagents

High purity acetonitrile was purchased from Burdick and Jackson (Baxter Healthcare Corporation, Muskegon, MI). Deionized water was from a NANOpure II (Barnstead Thermolyne, Dubuque, IA) water purification system. Benzaldehyde, 99%, benzoic acid, 99% and DSA (1-decanesulfonic acid sodium salt, 99%) were purchased from Aldrich Chemical Company, Milwaukee, WI. WY-23,096 [*N*-($\alpha$-phenylphenacyl)succinamic acid], benzoin (2-hydroxy-2-phenylacetophenone), WY-21,879 (benzoin hemisuccinate), WY-23,120 (4,5-diphenylimidazole-2-propionic acid), WY-22,075 (4,5-diphenyl-2-oxazolepropionamide), WY-20,910 (diphenylethanedione), oxaprozin (4,5-diphenyl-2-oxazolepropionic acid, also known as WY-21,743),

oxaprozin ethyl ester, tetraphenylpyrazine, and WY-23,027 (phenanthro[9,10-*d*]oxazole-2-propionic acid) were provided by Wyeth-Ayerst Laboratories, P.O. Box 8299, Philadelphia, PA 19101.

## Determination of Oxaprozin

The mobile phase consisted of aqueous solution (0.01M $KH_2PO_4$ - 0.005M DSA), methanol, and acetonitrile (2:1:1). The pH of the mobile phase was adjusted to 4.2 with phosphoric acid (85%). After mixing and pH adjustment, the mobile phase was filtered through a 0.22-$\mu$m Millipore filter and degassed by sonication under vacuum. Oxaprozin standard or sample was prepared to contain about 0.4 mg/mL in the mobile phase. All other related compounds were prepared to contain each about 0.04 mg/mL in the mobile phase. The liquid chromatograph was equipped with a 254 nm detector, and the flow rate was about 1 mL/min. As an example, the retention times for oxaprozin and several of its related compounds are shown in Table 1. The standard preparation was injected several times (10 $\mu$L) and the response was measured. The relative standard deviation for six replicate injections of the standard preparation is shown in Table 2. The sample preparation was injected, and the concentrations of oxaprozin and any of its related compounds were determined.

## Results and Discussion

Oxaprozin was analyzed and completely separated from its major related compounds and precursors using the above described HPLC method, as shown

## TABLE 1

### Limit of Detection and Regression Analyses

| | Retention Time (min) | Limit of Detection (μg/mL) | Correlation Coefficient (RS) |
|---|---|---|---|
| WY-23,096 | 5.9 | 0.060 | 1.0000 |
| Benzoin | 6.8 | 0.084 | 0.9997 |
| WY-21,879 | 9.5 | 0.084 | 0.9997 |
| WY-23,120 | 10.6 | 0.048 | 0.9997 |
| WY-22,075 | 12.5 | 0.048 | 0.9999 |
| WY-20,910 | 17.6 | 0.078 | 0.9990 |
| Oxaprozin | 19.8 | 0.054 | 0.9998 |
| WY-23,027 | 22.9 | 0.060 | 0.9999 |



## TABLE 2

### Precision and Ruggedness Analyses

| | Precision, RSD (%) n=6 | | Ruggedness, RSD (%) n=6 | |
|---|---|---|---|---|
| | Day 1 | Day 2 | Day 1 | Day 2 |
| WY-23,096 | 1.00 | 0.71 | 0.24 | 0.24 |
| Benzoin | 0.68 | 0.39 | 0.20 | 0.24 |
| WY-21,879 | 0.23 | 0.15 | 0.25 | 0.36 |
| WY-23,120 | 0.34 | 1.00 | 0.22 | 0.37 |
| WY-22,075 | 0.34 | 0.44 | 0.55 | 0.90 |
| WY-20,910 | 0.44 | 0.56 | 0.15 | 0.36 |
| Oxaprozin | 0.22 | 0.16 | 0.38 | 0.81 |
| WY-23,027 | 0.44 | 0.64 | 0.27 | 0.61 |

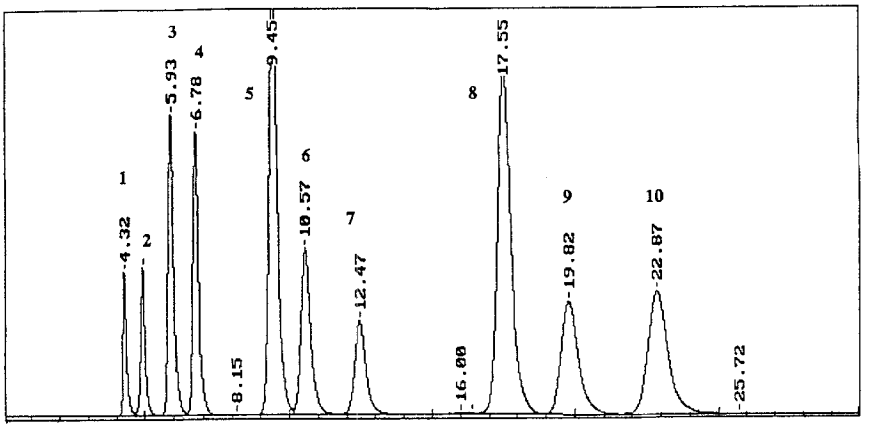


Fig. 1. Separation of oxaprozin from its potential related compounds and precursors: Benzoic acid (1), Benzaldehyde (2), WY-23,096 (3), Benzoin (4), WY-21,879 (5), WY-23,120 (6), WY-22,075 (7), WY-20,910 (8), Oxaprozin (9), and WY-23,027 (10). Column, 300 x 3.9 mm $\mu$ Bondapak $C_{18}$, flow rate 1.0 mL/min, detection at 254 nm.



in Fig 1. Photodiode array detection was used as evidence of the specificity of the method, and to evaluate the homogeneity of the peaks. Chromatographic peak purity at peak heart was determined using wavelength ratio comparison (224 nm vs 283 nm). This technique is applied to resolve coeluting peaks. The plot (Fig 2) showed that oxaprozin exhibited a homogeneous peak with no detectable impurities embedded in it. Also, complete resolution of oxaprozin from its related compounds, with good peak symmetry and no apparent shoulders, was demonstrated as shown in Fig 3.

Method precision and ruggedness were evaluated on oxaprozin and its major related compounds by two different analysts on two different days using different columns; the RSD's from each analyst (n=6) are listed in Table 2.

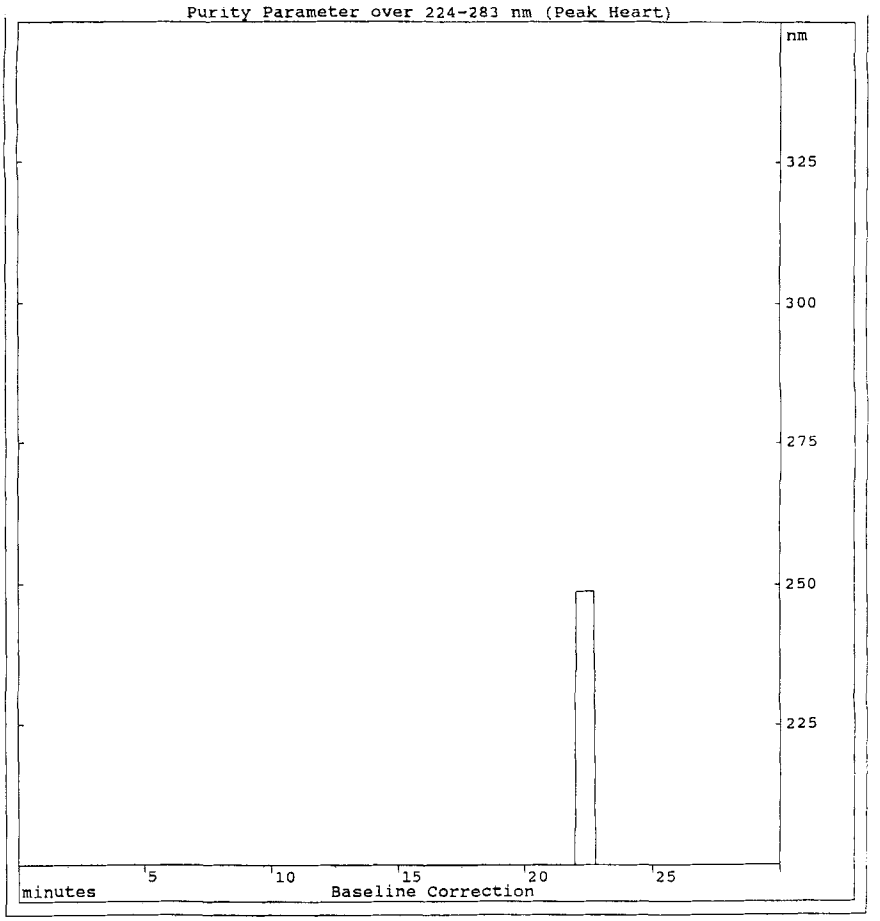


Fig. 2. Peak purity at peak heart for oxaprozin performed over 224 nm - 283 nm using diode array spectrophotometry.

Linearity of response was measured for oxaprozin and its major related compounds at 5 and 6 levels. The area count of each compound plotted against its consecutive concentration produced a straight line through the origin. Regression analysis yielded a correlation coefficient (RS) of 0.999 or better for

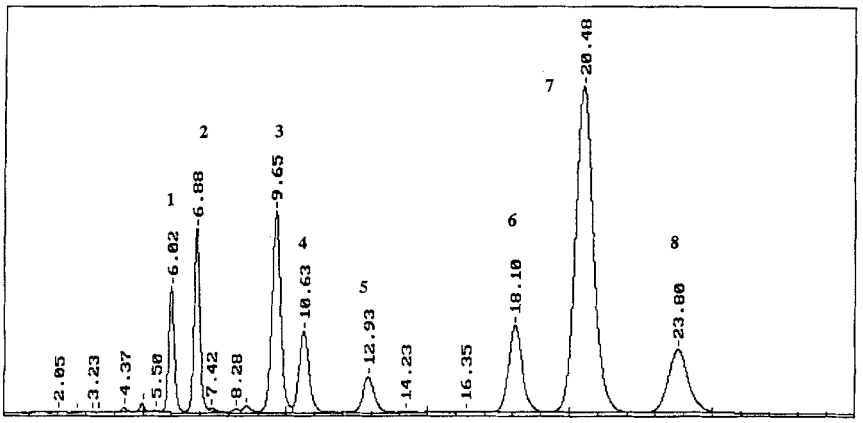


Fig. 3. Chromatogram of WY-23,096 (1), Benzoin (2), WY-21,879 (3), WY-23,120 (4), WY-22,075 (5), WY-20,910 (6), Oxaprozin (7), and WY-23,027 (8). Column, 300 x 3.9 mm $\mu$ Bondapak $C_{18}$, flow rate 1 mL/min, detection at 254 nm.



oxaprozin and its analogs, as can be seen in Table 1. These results showed a high degree of linearity in the range between 1.1 mg/mL to 0.4 mg/mL for oxaprozin, and between 0.08 mg/mL to 0.05 $\mu$g/mL for the other analogs.

The limit of detection was about 0.05 $\mu$g/mL for oxaprozin, and for its related compounds ranged from 0.05 $\mu$g/mL to 0.08 $\mu$g/mL (Table 1).

The ethyl ester of oxaprozin was also determined using the above described procedure, and its retention time was about 42 minutes when a flow rate of 1.5 mL/min. was used.

Tetraphenylpyrazine (TPP), which might be formed as a by-product during the synthesis of oxaprozin, is a symmetrical non-polar compound and was not eluted using the above HPLC method. However, using a modified mobile

phase consisting of 0.01 M $KH_2PO_4$, acetonitrile (1:9), pH 4.2, TPP was eluted and determined in oxaprozin samples. The retention time of TPP was 6.1 minutes, and that of oxaprozin was 3.1 minutes.

Accelerated degradation studies were performed to conform the validity of the method, also to demonstrate its specificity and to show that it can be stability indicating. Two separate solutions of standard oxaprozin (1 mg/mL in mobile phase) were prepared. One solution was exposed to ultraviolet (254 nm and 366 nm) light for 40 hours, and the other was kept at 50°C for two days. The degraded samples were then analyzed using the above HPLC method. Interestingly enough, oxaprozin exposed to UV light was mostly converted to WY-23,027, accompanied by some unidentified decomposition products, as shown in Fig 4. On the other hand, the heated oxaprozin solution showed several decomposition products, including three known related compounds (WY-23,096, WY-23,120 and WY-22,075), but no WY-23,027 was formed, as shown in Fig 5. Both degraded samples (UV and 50°C) were subjected to diode array analysis, and the more interesting photodegraded plot is shown in Fig 6. This plot report showed that oxaprozin (Rt = 22 min) and WY-23,027 (Rt = 26 min) had no detectable impurity peaks embedded in them and are free of co-eluting compounds. Their purity parameter values are not changing over the course of the peak and appear with a flat top. The earlier peaks, on the other hand, are bifurcated or show slope at

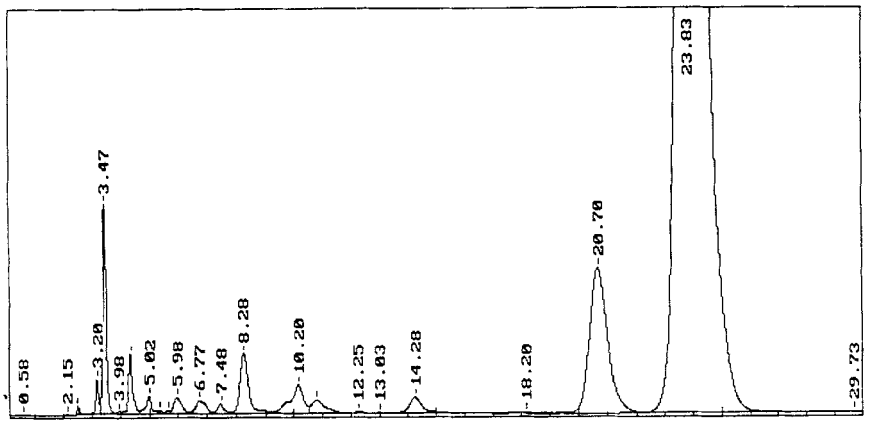


Fig. 4. Chromatogram of a solution of oxaprozin exposed to ultraviolet light. Column 300 x 3.9 mm $\mu$ Bondapak $C_{18}$, 1.0 mL/min, detection at 254 nm.



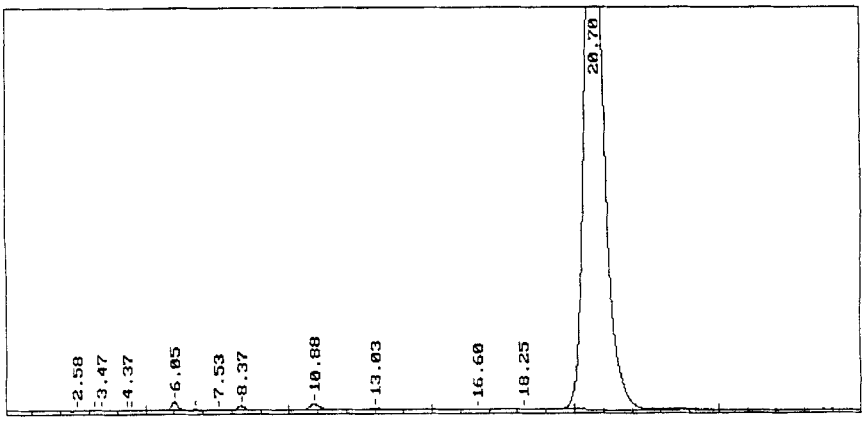


Fig. 5. Chromatogram of a solution of oxaprozin exposed to heat. Column, 300 x 3.9 mm $\mu$ Bondapak, $C_{18}$, flow 1.0 mL/min, detection at 254 nm.

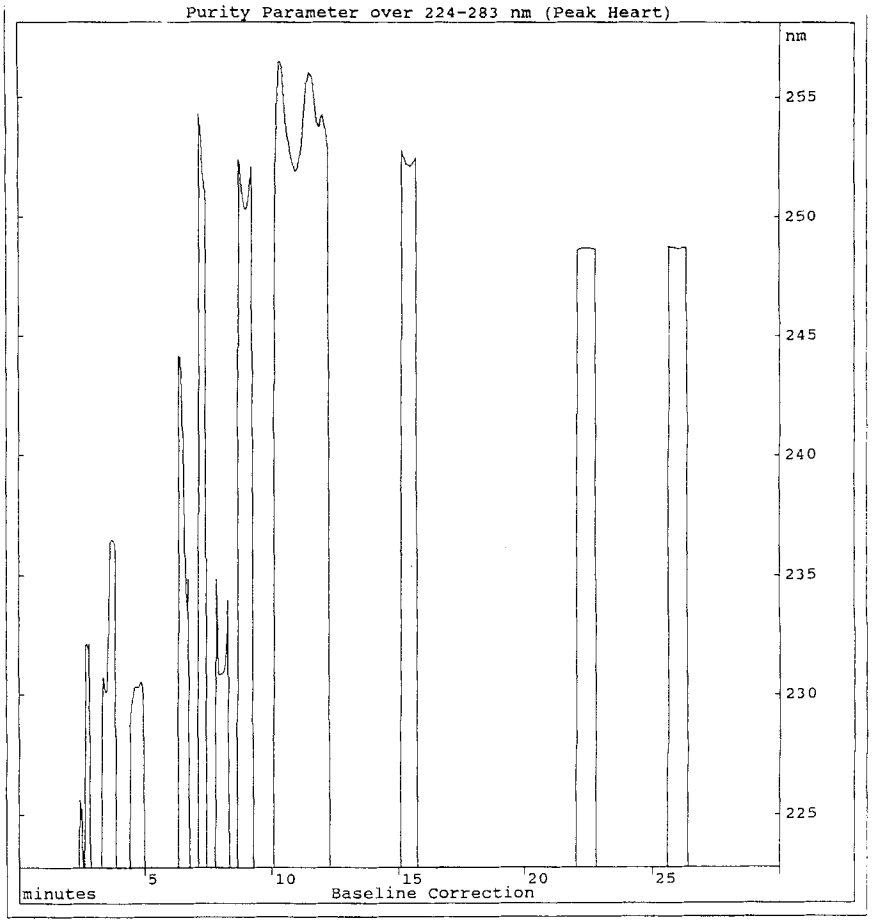


Fig. 6. Peak purity at peak heart for a photodegraded oxaprozin sample performed over 224 nm - 283 nm using diode array spectrophotometry.

top, indicating that their purity parameter values are not consistent across the width of the peak and hense the coelution of two (or more) substances (Fig 6).

This illustrates that the method can be used for determining the stability of oxaprozin in bulk pharmaceuticals. The method is also capable of detecting

intermediates and other related compounds which might be present at trace levels in finished products. Also, kinetic measurements can be carried out by this method to determine completion times for synthetic reactions.

## CONCLUSION

The above method provides a simple technique to separate and determine quantitatively oxaprozin from its precursors or possible related compounds in bulk pharmaceuticals. The method was rapid because no extraction was involved. Oxaprozin and several of its related compounds were quantitatively determined simultaneously in a single run. Furthermore, the method is stability-indicating, since several analogs and degradation products which might have been formed under the described stress conditions were completely resolved from oxaprozin (Figs 4 and 5) as shown by the photodiode array technique (Figs 2 and 6).



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