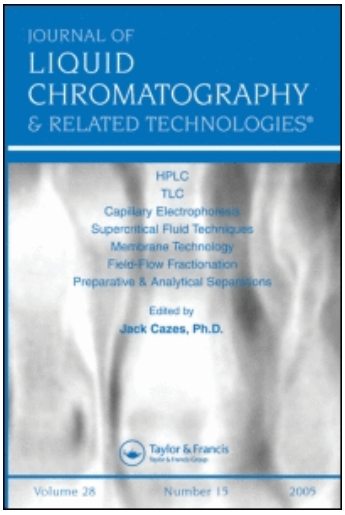

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SIMULTANEOUS HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF NON-STEROIDAL ANTI-INFLAMMATORY OXICAMS IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT

A simple and rapid isocratic high-performance liquid chromatographic method has been developed for the simultaneous analysis of the non-steroidal anti-inflammatory drugs tenoxicam, piroxicam, meloxicam, and lornoxicam using isoxicam as an internal standard. The analyte was chromatographed using a Lichrosphere RP18 column, a Tris acetic acid buffer-tetrabutylammonium reagent-tetrahydrofuran-acetonitrile as the mobile phase, and UV detection at 360 nm. The separation obtained was very good. The method was applied to pharmaceutical formulations containing a single active ingredient and was shown to be linear, sensible, accurate, and reproducible. A very small modification of the mobile phase enabled the separation of tenoxicam, piroxicam, isoxicam, cinnoxicam, meloxicam, and lornoxicam with symmetrical and well resolved peaks.

INTRODUCTION

Oxicams belong to a long-acting class of non-steroidal anti-inflammatory drugs (NSAIDs) which displays potent anti-inflammatory and analgesic activity and is effective in the treatment of rheumatoid arthritis, osteoarthritis, and degenerative joint diseases.[1] This enolic acid class of compounds (Figure 1) provides some drugs with variations in both efficacy and tolerability.

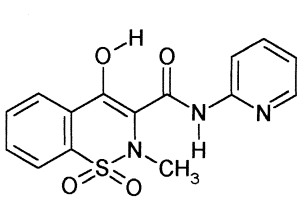
Searches in literature revealed that piroxicam (PIR) and tenoxicam (TEN) have been determined by means of a number of analytical procedures, almost exclusively based on high-performance liquid chromatography (HPLC) that permitted the assay of a compound alone or in the presence of expected metabolites in biological samples.[2-7] Only one report published an analytical method, also by HPLC, for plasma concentration measurement of meloxicam (MEL)[8] and lornoxicam (LOR).[9]

None of these papers gave information concerning the separation of related pharmaceutically important compounds of the oxicam class. However, only a few reports deal with the analysis of commercially available oxicams in their dosage forms and HPLC was rarely used;[10] spectrophotometric methods, especially, provided the analytical tool for the determination of PIR,[10-14] TEN,[15] and for stability studies of PIR.[14] A polarographic method was reported for the determination of TEN in pharmaceutical preparations.[1] A literature survey also showed that there was no published method for analysis of cinnoxicam (CIN).

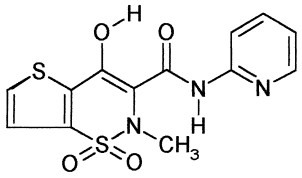
As a result, it was considered to be very useful to develop a selective method combining simple manipulation and use of standard equipment. That method would serve as a versatile analytical tool suitable for the simultaneous analysis of these drugs and would be of interest for quality control and clinical monitoring laboratories.

The similar characteristics of these compounds due to their closely related structure make difficult to distinguish them individually within their chemical class. HPLC appeared to offer the best approach,[2-9] which led us to regard this technique as appropriate for this purpose.

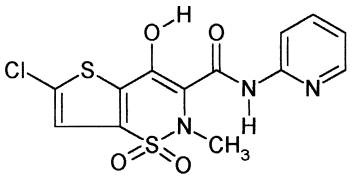
Therefore, in the present study, an isocratic HPLC method has been developed for a simultaneous analysis of five oxicams and is proposed for a rapid and reliable quality control of these drugs. It was tested for its suitability for the analysis of PIR, TEN, and MEL in pharmaceutical formulations. Isoxicam (ISO) marketing was suspended because of adverse effects: we used it in this work to serve as internal standard.



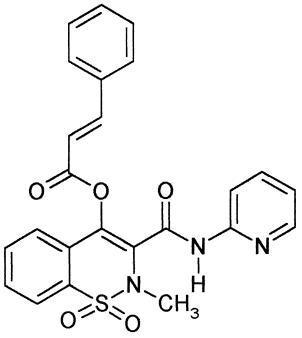
Piroxicam



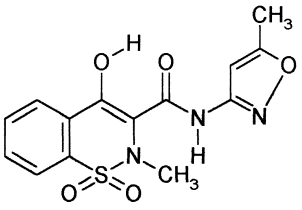
Tenoxicam



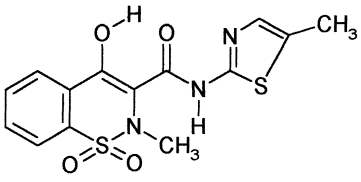
Lornoxicam



Cinnoxicam



Isoxicam



Meloxicam

Figure 1. Chemical structures of oxicams used in this study.

EXPERIMENTAL

Materials and Reagents

All chemicals were used without further purification. Anti-inflammatory agents used in this study were kindly supplied as follows: PIR, Pfizer (Orsay, France); TEN, Hoffmann-La Roche (Basel, Switzerland); ISO, Substantia (Orléans, France); MEL, Boehringer Ingelheim (Reims, France); LOR, Nycomed (Linz, Austria). CIN was extracted from commercial preparations. All solutions were filtered through a 0.45 μm nylon membrane (Sun International Trading, Wilmington, NC, USA) before injection. Tetrahydrofuran (THF) used was HPLC grade from C.I.L. (Ste Foy-la-Grande, France). Acetonitrile (ACN) Lichrosolv gradient grade and ortho-phosphoric acid (min. 85%) were from Merck (Nogent sur Marne, France). Tris (Trizma base Sigma ultra) and tetrabutylammonium hydroxide Fluka 40% solution in water (purissime for ionic chromatography) were purchased from Sigma Aldrich (St Quentin-Fallavier, France). 100% acetic acid Chromanorm for HPLC, diammonium hydrogen phosphate RP Normapur, and non stabilized 1,4-dioxan (min. 99.9%) Chromanorm for HPLC were obtained from Prolabo (Fontenay s/ bois, France). Water was deionized double distilled from a glass apparatus.

Apparatus and Chromatographic Conditions

The Thermo Separation Products HPLC system (TSP, Riviera Beach, FL, USA) employed consisted of a Model P100 solvent delivery pump equipped with a Model UV 150 variable-wavelength detector connected to a Model Data Jet integrator. The injector was a Model 7125 Rheodyne (Rheodyne, Cotati, CA, USA) with a 20 μL sample loop. The detector wavelength was set at λ = 360 nm and rise time at 0.5 sec. The integrator attenuation was fitted at 16 and the chart speed was maintained at 0.5 cm min^{-1}. The chromatographic separations were performed at ambient temperature on a stainless-steel Lichrocart cartridge (125x4mm I.D.). Lichrosphere 100-RP18, 5 μm particle size, from Merck (Nogent sur Marne, France). An Uptight stainless-steel precolumn (20x2 mm I.D.) fitted with Techopak 10C18 cartridge was used as a guard column. The mobile phase consisted of a mixture of buffer solution-tetrabutylammonium reagent-THF-ACN (705 + 15 + 70 + 210 by volumes). The buffer solution was a mixture of 0.05M Tris-0.05M acetic acid (15 + 85 v/v).

The tetrabutylammonium reagent was prepared by dissolving 8.8 g of diammonium hydrogen phosphate in about 50 mL of water, adding 25 mL of the tetrabutylammonium hydroxide solution. Then pH was adjusted to 7.5 by the

dropwise addition of phosphoric acid and the volume of the reagent made up to 100 mL with water. The aqueous solution of mobile phase had pH = 4.8. The mobile phase was filtered through Sartorius 0.2 μm Sartolon type filters (Sartorius, Goettingen, Germany) and sonically degassed prior to use at a flow-rate of 1.5 mL min^{-1}. It was prepared daily.

Standard Solutions

Stock solutions (100 mg L^{-1}) of oxicams were prepared in dioxane-water (3 + 1 v/v). The solutions were stored at 4°C and were found to be stable for at least one month. The working composite standard was prepared by combining an aliquot of each stock solution and diluting the mixture with the mobile phase. Working standard solutions were made by serial dilutions of stock solutions with incorporation of constant volume of the stock solution of ISO (internal standard) at a concentration of 5 mg L^{-1} and diluted with the mobile phase. These working solutions were freshly prepared each week and stored at 4°C. Five-point calibration curves at concentrations of 60, 80, 100, 120, and 140% of the analytical concentration were constructed for each drug. The calibration equations were calculated by least-squares linear regression of the peak-area ratio of the drug/internal standard (y) versus drug concentration (x).

Sample Preparation

All the studied compounds were not marketed. Samples of pharmaceutical products were obtained for three of these oxicams available on the market, TEN, PIR, and MEL.

Ten tablets, or the contents of ten capsules, from the sample to be analysed were accurately weighed and ground until just reduced to a fine powder. An accurately weighed amount of the powder equivalent to *ca.* 10 mg of oxicam was transferred into a 100 mL volumetric flask and extracted with *ca.* 90 mL of a dioxan-water (3 + 1 v/v) solvent system under sonication at room temperature for 20 min. The mixture was then diluted to volume with the extracting solvent and centrifuged at 5000 rev. min^{-1} for 5 min.

Depending on the drug present in the sample, an aliquot of the clarified solution was transferred into a volumetric flask in order to obtain a final concentration near the median zone of the calibration solutions; internal standard solution was added and the volume was adjusted with the mobile phase. A 20 μL volume of the resulting solution was injected onto the chromatograph.

Precision and Recovery

The precision of the proposed method was studied by assaying the samples several times using the expected concentrations. The intra-day variation was investigated by repetitive injections on the same day and the peak-area ratios (drug/internal standard) were averaged. Intermediate precision was determined by replicate analyses over a 3-day period.

Recovery experiments were carried out by adding known amounts of the appropriate standard to aliquots of ground tablets or capsules corresponding to 10 mg of oxicam and by analyzing them according to the proposed method. Fifty percent of the expected amount was added to the powdered sample. The samples were then treated as described under Sample Preparation.

RESULTS AND DISCUSSION

Selection of Sample Preparation Solvent

Oxicams generally are very poor water-soluble substances and exhibit poor solubility in organic solvents. Furthermore, data about solubilities of new oxicams such as LOR and CIN are not available. A single sample solvent for all products was considered desirable. Preliminary trials with methanol and acetonitrile in neutral, acidic, or basic medium were unsuccessful. The mixture dioxan-water (3 + 1 v/v) appeared to be a good solvent for the studied compounds. Its effectiveness was assessed statistically in the recovery assays.

Chromatographic Separation

The composition and the pH of the mobile phase were varied to optimize the chromatographic conditions. Some reports concerning the ionization behaviour of oxicams were published with contradictory results.[17-20] Oxicams are enolic acid compounds and because of their weak acidity, their HPLC determination is unsuitable by using reversed-phase chromatography with neutral eluent. Therefore, it was thought necessary to employ a buffer. We chose tris-acetic acid buffer for convenience in handling its mixtures with organic modifiers.

Our investigations indicated that separation of all oxicams could not be achieved with only ACN-buffer as mobile phase. The addition of tetrabutylammonium phosphate as counter-ion to the aqueous part of the eluent and the use of ACN and THF as organic modifiers resulted in satisfactory chromatography in terms of resolution and analysis time.

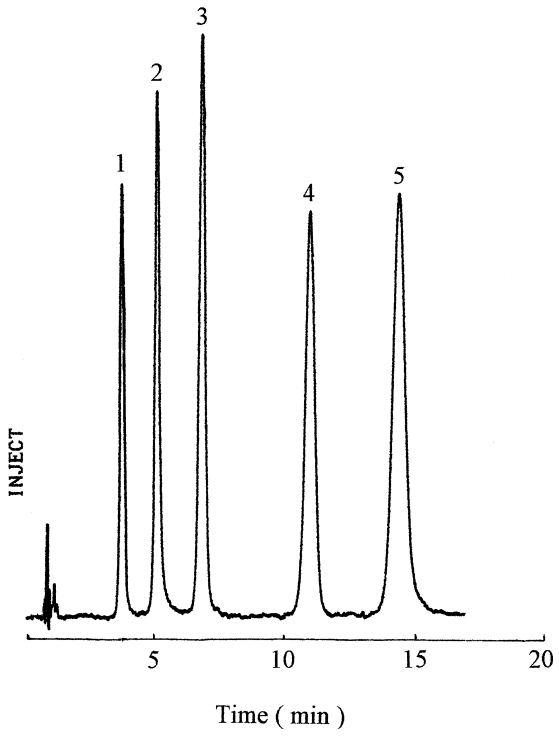


Figure 2. Representative chromatogram of the optimised separation of the oxicams investigated. Conditions: 125x4mm Lichrosphere RP18 (5μm) column; mobile phase, tris acetic acid buffer-tetrabutylammonium reagent-tetrahydrofuran-acetonitrile (705 + 15 + 70 + 210) at 1.5mL min^{-1}; detection at 360nm; injection 20μL. Peaks: 1=TEN; 2=PIR; 3=ISO (internal standard); 4=MEL; and 5=LOR.

Establishment of the proper pH of the aqueous part of the mobile phase was found to be quite important to be able to achieve efficiency needed for this application. Under pH 4.8 retention times of TEN and PIR slowly decreased, there was little change for MEL, whereas increased retention was observed for ISO, and especially LOR exhibited a rapid decrease in retention to such an extent that MEL and LOR would have overlapped.

A mobile phase pH above 4.8 slowly increased retention times of all studied compounds. THF was found to be effective in eliminating peak tailing. Under such conditions, the simultaneous determination of TEN, PIR, MEL, and LOR was possible, ISO being used as internal standard. A typical chromatogram is shown in Fig. 2.

Table 1

Retention Times, Detection, and Quantitation Limits for the Determination of Oxicams in Pharmaceutical Formulations*

Compound	Retention Time (Min.)	Detection Limit (ng Injected)	Quantitation Limit (ng Injected)
TEN	3.57	1.4	5
PIR	4.90	1.8	6
MEL	10.37	2.6	10
LOR	13.51	6	23
ISO	6.57		

* ISO = internal standard.

Table 2

Linearity Study with Standards for Determining Oxicam Concentrations in Pharmaceutical Samples[a,b]

Compound	Concentration Range (mg L^{-1})	Slope	Intercept	Correlation Coefficient
TEN	1.0 - 3.0	2.2560	-0.0046	0.9995
PIR	2.0 - 4.0	2.6436	-0.0315	0.9995
MEL	2.0 - 5.0	2.9914	-0.0188	0.9991
LOR	5.0 - 11.0	1.9041	-0.0187	0.9998

[a] ISO = internal standard. [b]Calculations based on 5 points for each drug.

The retention times are reported in Table 1. Under the described experimental conditions, the peaks corresponding to the studied compounds were well defined, well resolved and free from tailing, and chromatographic run was done within 14 min.

Linearity

All the calibration graphs displayed good linearity over the concentration ranges examined. For each plot, the slopes of the regression lines were in good agreement, intercept values were insignificant and the correlation coefficients were better than 0.999 (Table 2).

Table 3

Precision for the Determination of Oxicams in Pharmaceutical Formulations

Compound	Concentration (mg L^{-1})	Intra-day Precision[a] Peak-Area Ratio[c]	RSD (%)	Intermediate Precision[b] Amount Found[c]	RSD (%)
TEN	2	0.457 ± 0.006	1.31	1.993 ± 0.022	1.10
PIR	3	0.776 ± 0.005	0.64	2.949 ± 0.060	2.03
MEL (7.5mg tablet)	3.5	1.053 ± 0.008	0.76	3.517± 0.080	2.27
MEL (15mg tablet)	3.5	1.047 ± 0.01	0.96	3.570 ± 0.051	1.43

[a] (n = 5). [b](n = 3). [c] (Mean ± SD).

Table 4

Recovery Data for the Determination of Oxicams in Pharmaceutical Formulations[a]

Unspiked Amount Per Sample (mg)		Amount of Std Added (mg)	Found (mg)[b]	Recovery (%)	RSD (%)
TEN	10.4	5.1	15.20 ± 0.06	98.1	0.39
PIR	10.2	4.7	14.68 ± 0.13	98.5	0.88
MEL (15mg tablet)	9.5	5.6	14.96 ± 0.21	99.1	1.40
MEL (15 mg tablet)	10.4	5.2	15.70 ± 0.18	100.6	1.15

a (n=3); [b] (Mean ± SD).

Precision and Recovery

Results for intra-day and intermediate precision are shown in Table 3. The assays showed good precision within the same day and within different days as indicated by the relative standard deviation (RSD). The mean recoveries obtained by standard addition experiments ranged from 98.1 to 100.6% with a RSD < 1.5% (Table 4).

Table 5

Analysis of Pharmaceutical Formulations[a]

Compound	Dosage Form	Found[b]	Recovery (%)	RSD (%)
TEN	20 mg tablet	19.80 ± 0.42	99.0	2.12
PIR	10 mg capsule	9.83 ± 0.15	98.3	1.52
MEL	7.5 mg tablet	7.39 ± 0.08	98.5	1.08
	15 mg tablet	15.11 ± 0.38	100.7	2.51

[a] Mean of five determinations.
[b] (Mean ± SD).

Limits of Detection and Quantitation

Under the analytical conditions described, the limits of detection and quantitation defined as the smallest level of each analyte that provide a signal-to-noise ratio of 3 and 10, respectively are reported in Table 1.

Analysis of Commercial Samples

The proposed HPLC method was applied to the analysis of pharmaceutical formulations and the results obtained are summarized in Table 5. The quantities found were in conformity with the values claimed by the manufacturers.

For reasons of insurance, the manufacturer of CIN could not distribute his pure substance. We could get only corresponding commercial pharmaceutical forms that enabled us to investigate the separation of the six oxicams namely TEN, PIR, ISO, CIN, MEL, and LOR. The six oxicams were simultaneously separated by the same column, with the same apparatus, using a similar mobile phase containing less THF and more ACN, namely a mixture of buffer solution-tetrabutylammonium reagent-THF-ACN (715 + 15 + 50 + 220) at a flow-rate of 1.7 mL min^{-1}.

CIN was detected at 320 nm and the other five oxicams at 360 nm by programming the detector wavelength from 360 to 320 nm at 12.0 min and then back to 360 nm at 15.0 min. The other chromatographic conditions were not modified. The selectivity of the proposed method towards structurally related oxicam drugs is illustrated in Fig. 3.

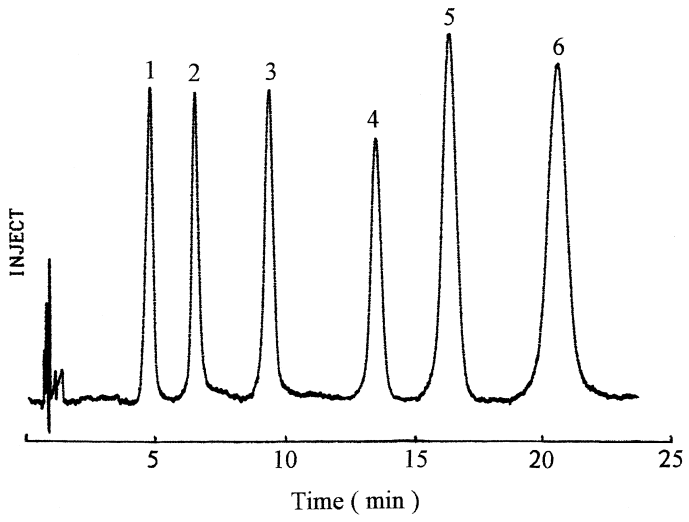


Figure 3. Chromatogram of six oxicams. 1=TEN; 2=PIR; 3=ISO; 4=CIN; 5=MEL; and 6=LOR. Chromatographic conditions as in text.

The six peaks were well resolved within 21 min. The retention times of TEN, PIR, ISO, CIN, MEL, and LOR were 4.71, 6.43, 9.26, 13.38, 16.18, and 20.37 min, respectively. Good resolution and peak shape should allow valid quantitation of CIN in comparison with the pure drug as for the other oxicams that we have analyzed.

CONCLUSION

The HPLC procedure reported here has proven to be simple, concise, and reproducible. The oxicam drugs could be completely separated as symmetrical peaks using THF-ACN-acidic aqueous eluent containing tetrabutylammonium reagent on a reversed-phase support. The combination of these modifiers was essential for satisfactory chromatography in terms of analysis time and resolution. The reproducibility and high separation power of the presented chromatographic method allowed adequate quantitative analysis of the oxicam drugs in pharmaceuticals. One of the advantages of this method is the use of one oxicam as internal standard for assay of the others. It may help to confirm or invalidate a difficult diagnosis of these structurally related compounds and can be applied in quality control laboratories for detection and quantitation of these NSAIDs.

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