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Quantitation of Flurbiprofen in Isopropyl Myristate by High Performance Liquid Chromatography

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고속액체크로마토그래피를 이용한 미리스틴산이소프로필중 플루르비프로펜의 정량

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An HPLC procedure with UV detection has been developed for the quantitation of flurbiprofen released into isopropyl myristate used as the receptor phase in an *in vitro* membraneless drug diffusion cell. The drug and the internal standard (oxaprozin) were extracted from isopropyl myristate with a mixture of dimethylsulfoxide:methanol:water (2:1:1) and quantitated using a reverse phase C_{18} column. The chromatograms were completeiy free from interfering peaks, and the relative retention times of flurbiprofen and the internal standard were 4.9 and 6.8 min, respectively. Calibration plots were linear over the concentration range of 1-200 μg/ml of flurbiprofen with correlation coefficients, all higher than 0.99. The mean intra-day precision and accuracy among three replicate sets of the assay in a day were 4.26 and 4.52%, respectively, whereas the mean inter-day precision and accuracy were 3.35 and 3.64%, respectively. The mean recovery of the drug was 92.5% over the calibration range. The method was simple, reliable and accurate for the quantitation of flurbiprofen in unpurified isopropyl myristate.

Keywords—flurbiprofen, isopropyl myristate, HPLC, assay validation

Flurbiprofen, a potent nonsteroidal anti-inflammatory drug, has been widely used for the treatment of rheumatoid arthritis and related conditions.[1,2] But, systemic side effects and gastrointestinal irritation are common due to the dose dumping effect after its oral administration.[3,4] Since it is usually administered over a prolonged period, it is important that these side effects of the drug should be reduced by any possible means while maintaining therapeutic drug concentration at its receptor site. Considering the fact that most inflammatory diseases occur locally under the skin, a topical application of flurbiprofen on the inflamed site can offer potential advantages of delivering a high drug concentration directly to the site of action and reducing systemic blood levels of the drug. This could not only lessen gastric irritation, but also reduce systemic side effects of the drug due to lower and sustained blood concentration after its topical application.[5,6] Based on such reasons, flurbiprofen gel using Pluronic F-127 (a polyoxyethylene surface-active block copolymer) was developed in our laboratory. To study the formulation factors of the gels on drug release, an *in vitro* diffusion cell without a barrier membrane has been empolyed and isopropyl myristate (IPM) was used as a receptor phase for the diffusing drug for the considerations of the immiscibility with the gel and its bipolar properties to mimic the biochemical composition of the skin. To

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monitor the release rate of flurbiprofen from the gel into IPM, it was necessary to develop an analytical procedure involving drug extraction from IPM and subsequent quantitation.

In this study, a simple, reliable and accurate HPLC assay method has been developed for the quantitation of flurbiprofen released into the IPM receptor phase. The method was successfully applied to study the release properties of flurbiprofen from various Pluronic topical gel formulation using the membraneless diffusion cell.

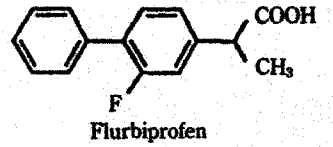
Experimental

Instrumentation

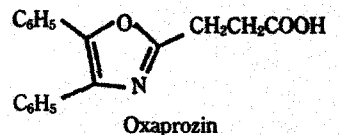
The chromatographic system consisted of a pump (Spectra-Physics, Model SP 8810), a UV detector (Spectra-Physics, Model Spectra Chrom 100), and an integrator (Spectra-Physics, Model SP 4270). A reciprocating shaker (Jeil Science) and a centrifuge (Vision Science) were used to extract the compounds from IPM. The column used was a μ-Bondapak C_{18} (Waters, 3.9×300 mm, 10 μm particle size) with a guard column (Upchurch C-130B).

Chemicals and Solvents

The following chemicals were used as received from suppliers without further purification: flurbiprofen, dimethyl sulfoxide (DMSO) and IPM (Sigma Chemical Co.), HPLC grade acetonitrile and methanol (Merck Co.), monopotassium phosphate (Ducksan Co.) and disodium phosphate (Sigma Chemical Co.). Oxaprozin was generously provided by Il-Dong Pharmaceutical Company. Water was distilled, deionized, and filtered in house.



Flurbiprofen



Oxaprozin

Figure 1—Molecular structure of flubiprofen and oxaprozin.

Chromatographic Conditions

The mobile phase consisted of 0.02 M phosphate buffer (pH 7.0) and acetonitrile in the volume ratio of 74:26 v/v%. The flow rate was 1.4 ml/min and the column temperature ambient. The UV detection was set at 254 nm with the detector range of 0.02 AUFS.

Standard Solutions of Flurbiprofen

A flurbiprofen stock solution of 200 μg/ml in IPM was prepared and serial dilutions of the stock solution were carried out with drug-free IPM to obtain flurbiprofen standard solutions of 1, 2, 5, 10, 20, 50, 100 and 200 μg/ml in IPM. The internal standard stock solution contained 20 μg/ml of oxaprozin in IPM. These solutions were found to be stable at least 1 month when stored in the refrigerator.

Extraction

Two hundreds μl of flurbiprofen standard solution were placed in a 2.0 ml volumetric flask, followed by the addition of 200 μl of the internal standard stock solution, and vortexed for 10 sec. After the volume was brought to 2 ml with the extraction medium consisting of DMSO:methanol: water (2:1:1). The sample was agitated on a reciprocating shaker for 15 min and centrifuged for 5 min at 2000 rpm. After discarding the IPM layer, 200 μl of the aqueous layer was transferred to a clean glass tube and diluted with an equal volume of the mobile phase. Fifty μl of the sample solution were injected onto the column for quantitation.

Calibration and Assay Validation

Calibration plots were obtained by assaying the standard solutions of flurbiprofen in IPM. The peak height ratios of flurbiprofen to the internal standard of each of the eight standard solutions were calculated and plotted as a function of flurbiprofen concentration. The obtained data were subjected to the regression analysis using the weight function of 1/(peak height ratio).

Precision and accuracy were evaluated over the entire range of the standard drug concentrations used. For the intra-day precision and accuracy studies, three replicate samples were prepared

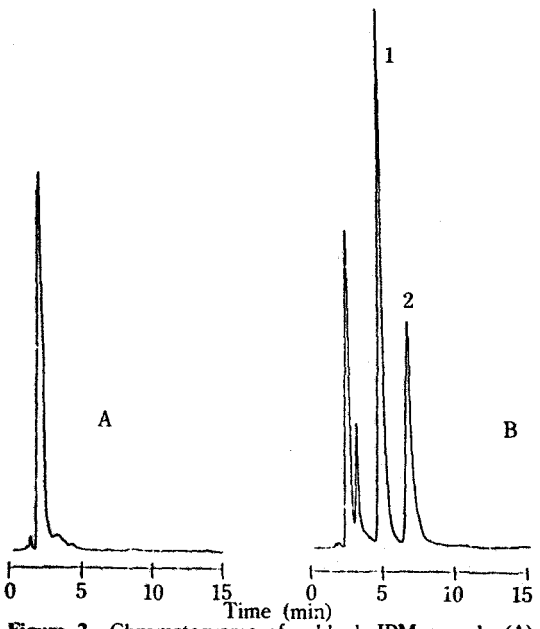


Figure 2—Chromatograms of a blank IPM sample (A), and an IPM sample (B) containing flubiprofen and the internal standard. Key: 1, Flurbiprofen; 2, Internal Standard.

and analyzed in a day for each of the eight different standard concentrations used. Similarly, for the inter-day precision and accuracy studies, the standard calibration assay was repeated on three different days over a period of one week and the results were statistically compared.

Results and Discussion

Chromatography

Representative chromatograms of the extracts of a drug-free IPM (A) and an IPM sample (B) containing flurbiprofen and the internal standard are shown on Fig. 2. No interfering peaks by the impurities in IPM are shown on the chromatogram of the extract of drug-free IPM using the extraction procedure described. The retention times for flurbiprofen and the internal standard were 4.9 and 6.8 min, respectively. The peaks were sharp and symmetrical, thus making the peak quantitation highly reliable.

Since IPM is miscible with nonpolar organic solvents, it was necessary to extract flurbiprofen from IPM using a polar solvent. DMSO offered an advantage of being a good solvent for flurbiprofen. But, IPM was still slightly soluble in DMSO. It was found that the mixture of DMSO, methanol, and water, in the volume ratio of 2:1:1, significantly reduced its solvent effect on IPM without changing its high extraction efficiency of the compounds. The addition of an equal volume of the mobile phase to the extract was necessary to obtain desirable chromatograms including the sharpness of peaks.

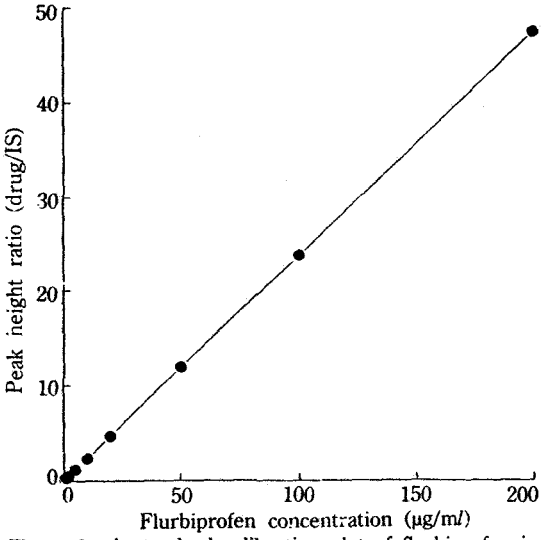


Figure 3—A standard calibration plot of flurbiprofen in IPM.

Calibration Plots

Fig. 3 shows one of the calibration plots obtained from the flurbiprofen standard IPM solutions containing 1-200 μl of flurbiprofen. The peak height ratios of flurbiprofen to the internal were plotted against flurbiprofen standard concentrations in IPM.

The linearity of the assay method was determined to show that a directly proportional relationship exists between the peak height ratios (Drug/IS) and the concentrations of the drug. To calculate the regression lines, a weighted linear regression analysis was used with a weight of 1/(peak height ratio). The use of the weighting factor generated a normal distribution of weighted residuals around the calibration plots over the en-

Table I—*Evaluation of the Intra-day Accuracy and Precision of Flurbiprofen Assay in Isopropyl Myristate.*

Spiked conc. (μg/ml)	Recovered conc. (μg/ml)	Absolute % deviation	CV %
1	0.9990[a]	0.10	0.99
	0.9946[b]	0.55	
	0.9803[c]	0.98	
2	2.0764	3.80	6.91
	2.0290	1.45	
	1.8198	9.01	
5	5.3149	6.30	0.94
	4.9974	0.05	
	5.3783	7.56	
10	11.0331	10.33	3.90
	10.0572	0.57	
	9.6291	3.71	
20	19.0722	4.64	7.03
	18.8313	5.84	
	18.7271	6.36	
50	48.5065	2.98	4.62
	54.2021	6.44	
	51.1443	2.28	
100	95.7201	4.28	6.28
	98.3987	1.60	
	107.7634	7.76	
200	204.0521	2.05	3.40
	200.1591	0.07	
	190.8901	4.55	
		4.52	4.26

[a]Assay 1: Conc=4.4084×ratio−2.1544
[b]Assay 2: Conc=4.2104×ratio−2.5783
[c]Assay 3: Conc=4.3548×ratio−1.3754

Table II—*Evaluation of the Inter-day Accuracy and Precision of Flurbiprofen Assay in Isopropyl Myristate.*

Spiked conc. (μg/ml)	Recovered conc. (μg/ml)	Absolute % deviation	CV %
1	0.9305[a]	6.95	4.17
	0.9181[b]	8.18	
	0.9918[c]	0.82	
2	2.1070	5.53	5.34
	2.1434	7.17	
	1.9369	3.15	
5	4.7614	4.77	1.95
	4.9008	1.98	
	5.3562	7.14	
10	10.0381	0.38	6.21
	9.8123	1.87	
	9.6558	3.44	
20	19.9043	0.48	2.04
	20.0762	0.38	
	19.3074	3.46	
50	54.3567	8.71	3.66
	51.3733	2.74	
	50.7982	1.59	
100	100.1419	0.14	2.77
	105.3321	5.33	
	104.8213	4.82	
200	195.2545	2.37	0.65
	192.8485	3.58	
	194.6811	2.66	
		3.64	3.35

[a]Day 1: Conc=4.2627×ratio−1.9089
[b]Day 2: Conc=4.6010×ratio−0.7658
[c]Day 3: Conc=4.1562×ratio−2.1141

tire range of drug concentration. The correlation coefficients for the regression lines, as shown in Tables I and II were, all higher than 0.99, indicating the excellent linearity of the calibration plots. The intercepts were almost passing through the origin.

Precision and Accuracy

Accuracy of the assay was evaluated using the spiked recovery method. The concentrations recovered were calculated by refitting the peak height ratios at each spiked standard concentration into the regression equation derived from the calibration data. The absolute % deviation at each spiked standard concentration was calculated as follows:

$$\text{Absolute \% deviation} = \frac{\text{Conc. recovered} - \text{Conc. spiked}}{\text{Conc. spiked}} \times 100$$

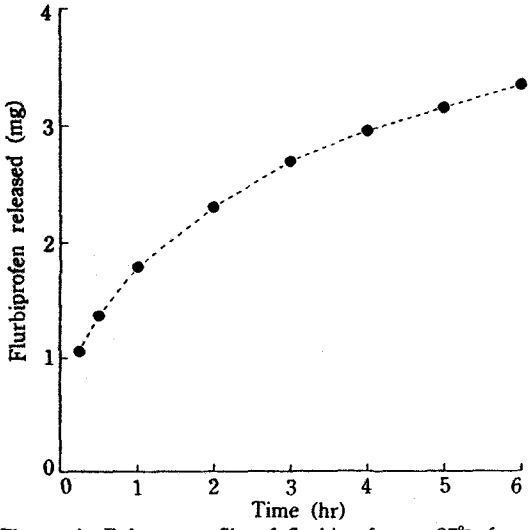


Figure 4—Release profile of flurbiprofen at 37℃ from a 20% Pluronic F-127 gel containing 1% flurbiprofen.

Calculated values were used as an index to evaluate the accuracy of the assay method.

Precision of the assay was determined in terms of a coefficient of variation (CV) among the concentrations recovered at each spiked standard concentration over the eight different standard concentrations used in the study.

Table I shows the intra-day precision and accuracy experiments for the flurbiprofen assay in IPM. Three replicate sets of spiked standard IPM solutions with eight different drug concentrations ranging from 1-200 μg/ml were analyzed in one day. Table II shows the inter-day precision and accuracy experiments for the assay. Each of the three replicate sets of spiked standard IPM solutions with eight different drug concentrations were analyzed on three different days over a period of one week.

The average intra- and inter-day precisions, expressed with % CV, were 4.26% (0.99-7.03%) and 3.35% (0.65-6.21%), respectively. The low % coefficients of variation indicate good reproducibility of the assay over the drug concentration range used in this study.

The average intra- and inter-day accuracies in terms of absolute % deviations were 4.52% and 3.64%, respectively. These low values indicate the reproducible and accurate measurement of the drug concentrations in IPM by the assay method described.

When the flurbiprofen concentration of 0.5 μg/ml of IPM was included in the calibration range and subjected to the same accuracy and precision evaluation, the % CV and absolute % deviation were all much higher than 10%.

Recovery

The assay recoveries of flurbiprofen from IPM were determined at each of the eight different standard concentrations of flurbiprofen used in the study. The peak heights of flurbiprofen obtained after extracting the spiked standard IPM solutions were compared with the peak heights of the equivalent amounts of flurbiprofen dissolved directly in the mixture of extraction medium and mobile phase (1:1).

The overall average recoveries of flurbiprofen calculated from the six calibration plots used for the intra-day precision and accuracy studies were 92.5%. The recovery of the internal standard was lower than that of flurbiprofen. However, oxaprozin was still valuable as the internal standard for this HPLC method since its recovery from IPM was very reproducible.

Sensitivity and Limit of Detection

The sensitivity of the assay was assessed with the limit of quantitation defined as the minimum concentration which can be quantitated with a statistically acceptable coefficients of variation. Since the acceptable CV was normally less than 10% for the quantitation of drugs in biological fluids, the lowest concentration used in this study, 1 μg/ml, which showed 4.17% CV in the inter-day precision study, was determined as the limit of quantitation of the assay. The limit of detection for flurbiprofen was 0.01 ng/ml based on 3 times signal to noise ratio.

Application

The HPLC method described is simple, reproducible and sufficiently sensitive enough for the quantitation of flurbiprofen in IPM. It is particularly useful in a situation where a large number of IPM samples are handled.

The assay has been sucessfully applied to the *in vitro* membraneless drug release studies of flur-

biprofen using IPM as a receptor phase. The release of flurbiprofen from a 20% Pluronic F-127 gel into the receptor phase is presented in Fig. 4. It can be seen that this simple and reproducible HPLC method which requires only one simple extraction procedure has enough sensitivity for the *in vitro* membraneless drug release model to evaluate flurbiprofen gel preparations.

Acknowledgement

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