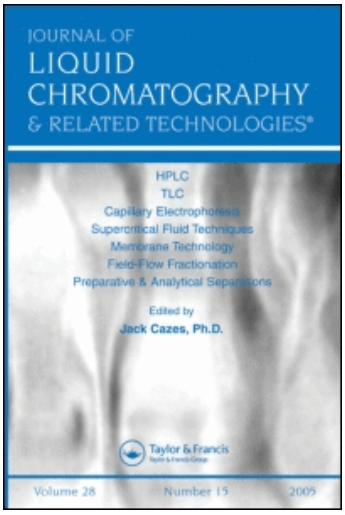

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OFEPIRIZOLE AND TIARAMIDE INPHARMACEUTICAL PREPARATIONS

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF EPIRIZOLE AND TIARAMIDE IN PHARMACEUTICAL PREPARATIONS

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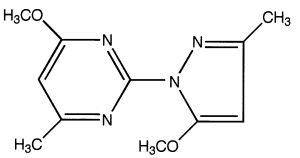
ABSTRACT

Rapid, accurate and reliable high performance liquid chromatographic methods for the determination of non-steroidal anti-inflammatory drugs epirizole and tiaramide in pharmaceutical preparations were developed using triethylamine, a modifying agent to the mobile phase. Extraction was carried out with methanol-water (1:1,v/v) after grounding tablet and granule preparations. The methods utilized reversed phase C18 column, UV monitoring at 250 nm, ethyl *p*-hydroxybenzoate as an internal standard for epirizole, and at 295 nm, methyl *p*-aminobenzoate as an internal standard for tiaramide. Regression analyses of three standard plots in concentration ranges of 0.05-0.4 mg/mL for epirizole and 0.18-1.44 mg/mL for tiaramide gave respective correlation coefficients >0.99998 and >0.99997. Relative standard deviations of the slopes were 0.180% and 0.888%, respectively. Percentage recoveries of these compounds for four commercially available drugs ranged between 99.06 and 103.14 and between 99.15 and 99.71 of the labeled amounts of epirizole and tiaramide, respectively. The methods were successfully applied to determine contents of epirizole and tiaramide in marketed drugs.

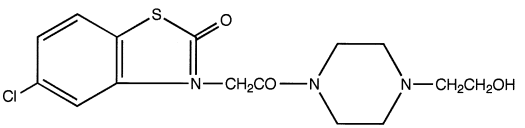
INTRODUCTION

Epirizole [4-methoxy-2- (5-methoxy-3-methyl-1-pyrazolyl)-6-methyl-pyrimidine][1] and tiaramide hydrochloride [2-(5-chloro-2-oxobenzo (1,3) thiazolin-3-yl)-N-(4-(2-hydroxyethyl)-1,4-diazacyclohexyl) acetamide monohydrochloride][2] are non-steroidal anti-inflammatory drugs (Figure 1) currently widely used for the treatment of musculoskeletal disorders and inflammation in Japan.[3]

In spite of wide-spread use of these drugs, only a few procedures for the analysis and measurement of these drugs have been described.[4-8] To the best of our knowledge, there is only one method for the determination of epirizole in pharmaceutical dosage forms by high performance liquid chromatography (HPLC),[4] and only a few methods for the determination of tiaramide in biological fluids by gas chromatography[5] and HPLC,[6] and in pharmaceutical preparations by UV spectrophotometry[7] and HPLC.[8] However, the HPLC method[6] was performed using an ODP column with a mobile phase containing salt modifiers undesirable to column maintenance.



Epirizole



Tiaramide hydrochloride

Figure 1. Structures of epirizole and tiaramide.

Triethylamine, a modifying agent to the mobile phase, was evaluated as a competing base for the retention control and peak shape improvement in the reversed phase HPLC analysis of selected basic drugs.[9-12] Its effect is due to the shielding of free silanol groups in silica based columns, which otherwise strongly interact with especially basic analytes.[13] In addition to their ability to reduce peak tailing, triethylamine is also useful as selectivity-enhancing agents.[14]

On the basis of the information regarding to the influence of this amine modifier, we tried to develop simple, rapid and reliable HPLC methods for routine and accurate determination of the drugs epirizole and tiaramide in pharmaceutical preparations.

The newly developed HPLC methods were applied to four commercial preparations marketed in Japan to evaluate the accuracy of the methods.

EXPERIMENTAL

Chemicals and Reagents

Epirizole and tiaramide hydrochloride for determination were of the Japanese Pharmacopoeia, thirteenth edition (JP XIII) quality. Epirizole contained not less than 99.0% of $C_{11}H_{14}N_4O_2$ (mol. wt.: 234.26), when dried. Tiaramide hydrochloride contained not less than 99.0% of $C_{15}H_{18}ClN_3O_3S.HCl$ (mol. wt.: 392.31), when dried. Ethyl *p*-hydroxybenzoate, triethylamine and HPLC-grade acetonitrile were purchased from Wako Pure Chemical Industries (Osaka, Japan). Methyl *p*-aminobenzoate was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). All other reagents were of analytical grade.

Apparatus

A Shimadzu (Kyoto, Japan) HPLC system was used consisting of the following components: Model LC-6A pump, a model CTO-2A column oven, a model SPD-6A UV detector, and a model C-R6A integrator/recorder equipped with a Rheodyne 7125 sample injection valve (20 μL). Column temperature was 40°C and chromatographic separation was carried out on Wakosil 5C18 column (5 μm, 150 × 4.6 mm I.D., Wako Pure Chemical Industries, Osaka, Japan). An ultrasonic bath (model B-42H, Branson Co, Shelton, CT, USA) was used to dissolve the samples in methanol-water (1:1, v/v).

Standard Solutions

Standard stock solutions of epirizole (2.5 mg/mL), of tiaramide (9.1 mg/mL) and their respective internal standard solutions of ethyl *p*-hydroxyben-

zoate (1.8 mg/mL) and methyl *p*-aminobenzoate (0.7 mg/mL) were prepared by dissolving appropriate amounts of respective compounds in methanol-water (1:1, v/v) at room temperature.

Chromatographic Conditions

After a series of preliminary experiments based on our previous experiences[4, 8] regarding the isolation of compounds which were chemically related to epirizole and/or tiaramide, the following mobile phases were used in this study.

The mobile phase for epirizole was a mixture of water, acetonitrile, triethylamine (750:250:1, v/v), and water, acetonitrile, triethylamine (800:200:1, v/v) for tiaramide, each of which was adjusted to pH3.0 with phosphoric acid. Flow-rate was 1.0 mL/min and 0.8 mL/min for epirizole and tiaramide, respectively. The column effluent was monitored for epirizole at 250 nm and for tiaramide at 295 nm using a detector range of 0.32 absorbance unit of full scale (AUFS) and a chart speed of 1 mm/min. An aliquot of stock solution (5 μL) was injected onto the analytical column with a manual HPLC injector.

Calibration Assay

Firstly, 0.5, 1, 2, 3, 4 mL of the epirizole or tiaramide standard stock solution were pipetted into 25 mL volumetric flasks, followed by the addition of 2.5 mL of the respective internal standard solutions to the corresponding flasks and then made with methanol-water (1:1, v/v) up to the mark. An aliquot (5 μL) of each solution was then injected onto the analytical column. All measurements were performed in duplicate for each concentration in three different days over one week.

The peak areas were measured and the peak area ratios of analyte to internal standard were then plotted against the respective concentration of epirizole and tiaramide. A least square linear regression analysis was used to determine the slope, Y-intercept, and the correlation coefficients of the standard plots.

Sample Preparation

Ten tablets or 1 g weighed granules were finely ground and mixed well in a mortar. An accurately weighed powdered sample containing 20 mg of epirizole or 80 mg of tiaramide was transferred to a 100 mL volumetric flask. Ten mL of the internal standard solution for epirizole (ethyl *p*-hydroxybenzoate) or for tiaramide (methyl *p*-aminobenzoate) was added to the corresponding flask, followed by swirling by hand for 2-3 min.

The volume was adjusted with methanol-water (1:1, v/v) to 100 mL and sonicated for 30 min in an ultrasonic bath. The resulting solution was then filtered through a 0.5 μm PTFE filter (Toyo Roshi Kaisha, Tokyo, Japan). An aliquot (5 μL) of each filtered-solution was injected onto the column. Four different types of commercially available pharmaceutical dosage forms (50 mg/tablet, 300 mg/g granules of epirizole, and 50, 100 mg/tablet of tiaramide) were analyzed for statistical evaluation of the assay.

RESULTS AND DISCUSSION

Chromatography

A reversed phase HPLC procedure was developed as a suitable method for the analysis of epirizole and tiaramide in pharmaceutical preparations. The chromatographic conditions were adjusted in order to provide a HPLC procedure capable of separating epirizole and tiaramide, and their respective internal standards ethyl *p*-hydroxybenzoate and methyl *p*-aminobenzoate. In the analyses of these compounds, we had found that there was an unsymmetrical and tailed epirizole peak without triethylamine, and there was no tiaramide peak without triethylamine. We also found that pH and acetonitrile concentration of the mobile phase substantially affected the retention of epirizole and tiaramide. Accordingly, we examined the effect of pH of the mobile phase with the pH range between 2.5 and 7.5, and found that the retention of tiaramide was weaker than that of its internal standard with pH smaller than 3.5; whereas that of epirizole was virtually not affected (Figure 2). As a result of the observations, we had selected pH3.0 as optimal. In order to determine an optimal concentration of acetonitrile in the mobile phase, we examined the acetonitrile concentration between 10 and 40%, and found that the retention time of epirizole and tiaramide became shorter with the increasing concentration of acetonitrile (Figure 3). According to these observations, we had selected the acetonitrile concentration of 25% for epirizole and 20% for tiaramide so as to assure complete separation of these compounds within 20 min. After several series of these preliminary experiments, a mixture of water, acetonitrile, and triethylamine (750:250:1, v/v) adjusted to pH3.0 with phosphoric acid, at a flow-rate of 1.0 mL/min with UV detection at 250 nm was found to be an optimal condition allowing an adequate separation of epirizole and the internal standard. A mixture of water, acetonitrile, and triethylamine (800:200:1, v/v) adjusted to pH3.0 with phosphoric acid, at a flow-rate of 0.8 mL/min with UV detection at 295 nm was found to be an optimal condition allowing an adequate separation of tiaramide and the internal standard. Figures 4 and 5 show typical HPLC chromatograms of epirizole (0.2 mg/mL) and tiaramide (0.72 mg/mL) standard solutions, respectively. Epirizole, tiaramide, and their respective internal standard ethyl *p*-hydroxybenzoate and methyl *p*-aminobenzoate were separated very well with respective retention times of 8.6 min, 8.2 min, and 16.2 min, 12.5 min. The resolution (*Rs*) between the peaks for epirizole and its internal standard was 10.1, and for

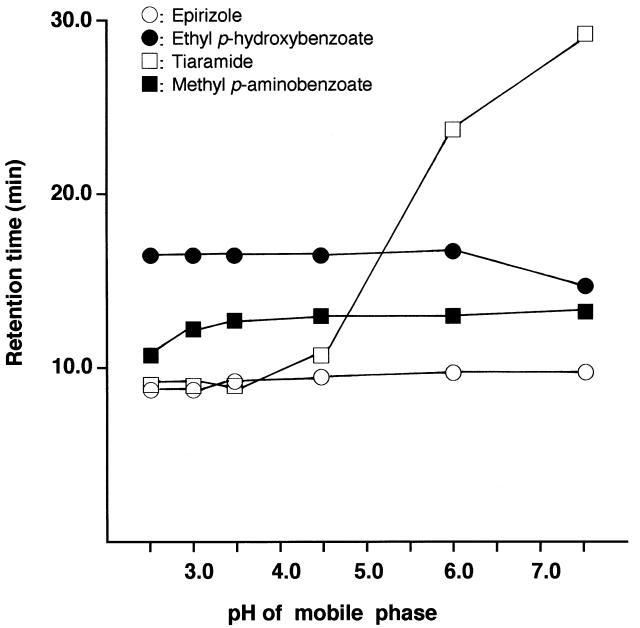


Figure 2. Effect of pH of the mobile phase on retention times of epirizole and its internal standard ethyl *p*-hydroxybenzoate, and tiaramide and its internal standard methyl *p*-aminobenzoate.

tiaramide and its internal standard was 5.7. The relative standard deviation (RSD) for six replicate injections of the standard preparation was 0.16% for epirizole and 0.70% for tiaramide, respectively.

Linearity and Precision

For all of these four compounds, sharp and symmetrical peaks were obtained with good baseline resolution and minimal tailing, thus facilitating the accurate measurement of the peak area ratios. Calibration plots for the peak area ratios of varying amounts of epirizole (0.05-0.4 mg/mL) and tiaramide (0.18-1.46 mg/mL) to the constant amounts of respective internal standards ethyl *p*-hydroxybenzoate (0.18 mg/mL) and methyl *p*-aminobenzoate (0.07 mg/mL) were highly linear, and the regression analyses of the data gave the respective slopes and intercepts. Three duplicate analyses of epirizole and

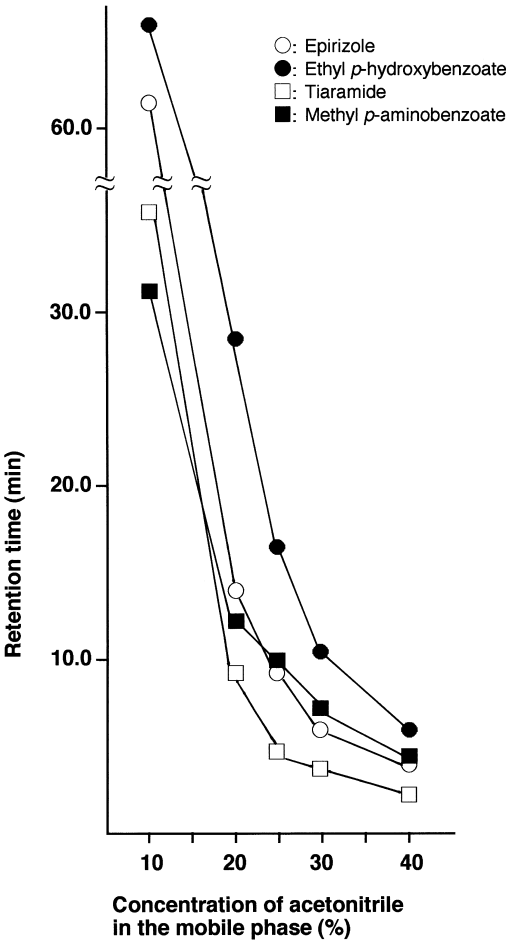


Figure 3. Effect of the concentration of acetonitrile in the mobile phase on retention times of epirizole and its internal standard ethyl *p*-hydroxybenzoate, and tiaramide and its internal standard methyl *p*-aminobenzoate.

tiaramide were conducted on three different days over a one-week period. The results of this evaluation are summarized in Tables 1 and 2.

The average correlation was 0.99998 and the RSDs of the slopes of the each of three lines were 0.18% and 0.89%, respectively. The results, thus confirmed the excellent linearity of the calibration plots and high reproducibility of the assay.

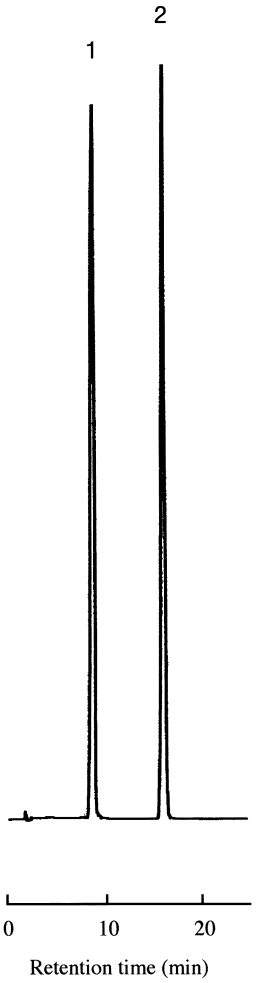


Figure 4. Liquid chromatogram of epirizole. Column: Wakosil 5C18; mobile phase: a mixture of water, acetonitrile, triethylamine (750:250:1, v/v) adjusted to pH3.0 with phosphoric acid; flow-rate: 1.0 mL/min; UV detection: 250 nm. Peaks: 1=epirizole (0.2 mg/mL), 2=internal standard ethyl *p*-hydroxybenzoate (0.18 mg/mL).

Method Application

The newly developed method for the analysis of these compounds was applied to measure the concentration of epirizole in commercially available dosage forms of three tablets and one granules preparation, and that of

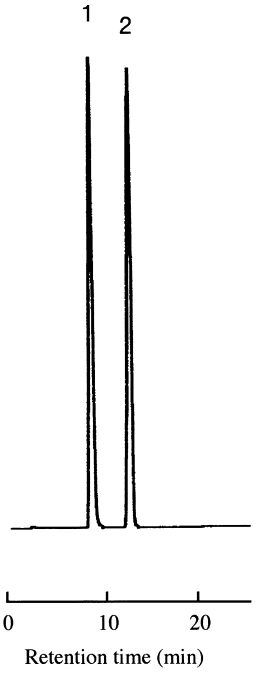


Figure 5. Liquid chromatogram of tiaramide. Column: Wakosil 5C18; mobile phase: a mixture of water, acetonitrile, triethylamine (800:200:1, v/v) adjusted to pH3.0 with phosphoric acid; flow-rate: 0.8 mL/min; UV detection: 295 nm. Peaks: 1=tiaramide (0.73 mg/mL), 2=internal standard methyl *p*-aminobenzoate (0.07 mg/mL).

Table 1

Regression Analyses of Three Standard Plots of Epirizole*

Standard	Slope	Intercept	Correlation Coefficient
No. 1	1.044141	-0.002808	0.999993
No. 2	1.044971	-0.001979	0.999990
No. 3	1.047739	-0.003586	0.999985

* These results were obtained from the measurements in three different days over one week calculated from the means of duplicate determination of five different concentrations (0.05, 0.1, 0.2, 0.3, 0.4 mg/mL).

Table 2

Regression Analyses of Three Standard Plots of Tiaramide*

Standard	Slope	Intercept	Correlation Coefficient
No. 1	0.266740	+0.002263	0.999992
No. 2	0.269253	+0.002985	0.999996
No. 3	0.271518	+0.004163	0.999978

* These results were obtained from the measurements in three different days over one week calculated from the means of duplicate determination of five different concentrations (0.18, 0.36, 0.73, 1.09, 1.46 mg/mL).

tiaramide in four commercially available tablets. The powdered samples were prepared by finely grounding in mortar as described above. No interfering peaks due to pharmaceutical excipients were found in chromatograms of all preparations.

Table 3 shows results of the measurements of epirizole using the methods described above as a percentage of claimed content and the RSD of epirizole measurements for each pharmaceutical preparation. The RSD values were obtained by repeating the measurements five times for each sample. The per-

Table 3

Determination of Epirizole in Pharmaceutical Preparations*

Sample	Pharmaceutical Forms	Claimed Content	Percentage of Claimed Content (Mean ± SD)	Relative Standard Deviation (%)
No. 1	Tablets	100[a]	103.14 ± 0.61	0.588
No. 2	Tablets	50[a]	99.06 ± 0.82	0.826
No. 3	Tablets	50[a]	99.97 ± 1.05	1.053
No. 4	Granules	300[b]	99.30 ± 0.79	0.795

[a] mg/tablet. [b] mg/g granules. These results were obtained by measuring five samples from one pharmaceutical preparation (each measurement was done duplicate). The relative standard deviation means the coefficient of variation.

Table 4

Determination of Tiaramide in Pharmaceutical Preparations*

Sample	Pharmaceutical Forms	Claimed Content	Percentage of Claimed Content (Mean ± SD)	Relative Standard Deviation (%)
No. 1	Tablets	100[a]	99.51 ± 0.52	0.522
No. 2	Tablets	100[a]	99.44 ± 1.12	1.128
No. 3	Tablets	100[a]	99.71 ± 1.20	1.211
No. 4	Tablets	50[a]	99.15 ± 0.45	0.452

[a] mg/tablet. * These results were obtained by measuring five samples from one pharmaceutical preparation (each measurement was done duplicate). The relative standard deviation means the coefficient of variation.

centages of claimed contents or the mean recoveries and the RSDs were 99.06-103.14% and 0.588-1.053%, respectively.

Table 4 shows results of the measurement of tiaramide as the percentages and the RSDs of the claimed contents. The percentages of claimed contents or the mean recoveries and the RSDs were 99.15-99.71% and 0.452-1.211%, respectively. These results confirmed the excellent precision and accuracy of the newly developed method for the analysis of these compounds in the pharmaceutical preparations.

CONCLUSIONS

The newly developed HPLC methods presented in this paper was demonstrated to have the advantages of simplicity, precision, and convenience for the determination of epirizole and tiaramide. The methods also allow a direct determination of these compounds, by eliminating several tedious steps involved in other assay methods. The methods reported here will, therefore, be useful for routine analytical and quality control assays of epirizole and tiaramide in pharmaceutical preparations.

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