## Research Article

# Modification and Validation of an HPLC Method for Quantification of Piroxicam

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## Abstract

Piroxicam is a NSAID that is widely used in the treatment of joint pain and osteoarthritis. The objectives of the study were to modify and validate HPLC method so as to obtain an accurate, sensitive and precise method to quantify piroxicam concentrations without interference from the other ingredients presence in the formulation. The method published by Owen *et al*. was adapted and modified to suit the above requirements. The modification was carried out on the mobile phase as the mobile phase used by the authors was not able to separate the drug peak from the interference of the formulation excipients. The modified mobile phase consisted of 5 mM of disodium hydrogen phosphate adjusted to pH 3 with concentrated ortho phosphoric acid, methanol, acetonitrile and glacial acetic acid at ratios of 27:20:52:1 respectively. The method was validated and found to be specific, precise, accurate and reproducible even when run at different times of the same day or on different times on different days. The limit of detection and quantification were determined to be 0.035 µg/ml and 0.0625 µg/ml respectively. It could be concluded that this method could be used to determine piroxicam concentration in the samples collected from *in vitro* study of permeability through the synthetic membrane and excised rat skin.

**Keywords:** Piroxicam, HPLC, Quantification analysis, Modification.

## Introduction

Piroxicam is a NSAID that is widely used in the treatment of joint pain and osteoarthritis. Many HPLC method were reported in the previous literature to measure piroxicam in plasma samples [2,3]. To detect piroxicam being transferred through cellulose membrane and skin in *in- vitro* franz diffusion cell, a method of high sensitivity is required. Because it is expected that piroxicam quantity diffused into the receptor cell is very low. The method published by Owen *et al*. (1987) was adapted and modified to suit the above requirements. The modification was carried out on the mobile phase as the mobile phase used by the authors was not able to separate the drug peak from the interference of

the formulation excipients. These excipients are the constituents of nano-cream. These are palm oil esters, Tween 80, Span 20 and phosphate buffer as external phase. Additionally, the interference of constitutes that might be leached from the skin during the diffusion process.

The objectives of the study were to modify and validate HPLC method so as to obtain an accurate, sensitive and precise method to quantify piroxicam concentrations without interference from the other ingredients presented in the formulation and other leachable skin constituents.

## Materials and Methods

### Materials

Disodium hydrogen phosphate was supplied by R & M Chemicals (UK). Orthophosphoric acid and glacial acetic acid were supplied by BDH (UK). Methanol and acetonitrile (HPLC grade) were purchased from J.T. Baker (USA). Nylon membrane filters (0.45µm pore size) was purchased from Whatman International (UK).

### Methods

### Instrumentation

The Shimadzu HPLC system (SCL-10A) consisted of an isocratic pump (Shimadzu Co, Japan, model LC-10-ATVP), an auto sampler (Shimadzu, Japan, model SIL-10AD) and a model SPD-10AV UV-VIS detector. The reverse phase chromatography was performed with an analytical stainless steel column (150 mm × 2.0 mm), containing C18 stationary phase 5-µm (thermo, Germany) protected with a SecurityGuard™ cartridge system (Phenomenex) and a 0.45-µm in-line filter.

### Chromatographic conditions

The mobile phase consisted of 5 mM of disodium hydrogen phosphate  adjusted to pH 3 with concentrated ortho phosphoric acid, methanol, acetonitrile and glacial acetic acid at ratios of 27:20:52:1 respectively. The mobile phase was filtered through 0.45 µm nylon membrane filter and degassed prior to use. The mobile phase used

by Owen *et al.* (1987) was a mixture of 0.03% phosphoric acid (pH 2.5):acetonitrile at a ratio of 55:45. Samples were placed in the auto-sampler. 20 µg was injected and eluted through the separation column at a flow rate of 1 ml/min, at room temperature and detected by UV-VIS at a wavelength of 350 nm.

### Preparation of stock and working standard solutions

Piroxicam stock solution of 1 mg/ml was prepared by dissolving appropriate weight of piroxicam in acetonitrile and stored at 4 °C. The stock solution prepared was consequently diluted with acetonitrile to obtain working standard solution of 25 µg/ml when needed.

### Preparation of calibration standards

The working standard solution of piroxicam was serially diluted with the mobile phase to produce concentrations of 0.0625, 0.125, 0.25, 0.5, 1 and 2 µg/ml.

### Method validation

### Linearity

The linearity of standard curve for the concentrations range 0.0625-2 µg/ml of piroxicam was determined by using freshly prepared calibration standard samples. Six linearity curves were analysed. Each calibration curve consisted of six concentrations as in 2.2.4. The samples were quantified by measuring the peak height corresponding to piroxicam. The calibration curve was a plot of piroxicam concentration in calibration standard against the corresponding piroxicam peak height of HPLC chromatograms. A correlation of more than 0.999 was recommended for all the calibrated curves.

### Specificity

Samples of blank formulation of E16 which contains all ingredients but without piroxicam and samples of E16 containing piroxicam were analysed by the same method. Weighed amount of the samples were dissolved in the mobile phase and then diluted with the mobile phase.

The samples were analysed to quantify the strength of formulation E16 prepared and assess any interference by other ingredients of the formulation at the retention time of piroxicam.

**Precision and accuracy**

The precision and accuracy of the mean were determined for the inter-day and intra-day analysis to validate the accuracy and precision of the method. For inter-day validation, six sets of samples at six different concentrations were evaluated for six different days by assessing the precision and accuracy. For intra-day validation, six sets of samples at six different concentrations were analysed on the same day and evaluated by assessing the precision and accuracy. Precision must not be above ±15% for calibrated concentrations level and not more than ±20% for lowest value in the calibration curve [4]. Similarly, accuracy of the mean should not be more than ±15% at all concentrations levels and not more than ±20% for lowest value in the calibration curve.

Precision was calculated according to the following equation:

(Standard deviation/ mean) ×100

Accuracy was measured by the following equation:

(Nominal concentration/ actual concentration)×100

**Limit of detection and limit of quantification**

The limit of detection was defined as the concentration that exhibit HPLC peak measurement of three times the level of baseline noise, in other words, at a signal to noise ratio of 3:1. The lowest concentration defined as the limit of quantification must have a peak height value of at least equal to five times the baseline noise.

# Results and Discussion

## Linearity

The calibration curve (Fig. 1.1) exhibited an excellent linearity over the concentration range of 62.5-2000 ng/ml. The mean linear regression equation from six replicated calibration curve were $y = 2031.1x - 1.495$, with a correlation coefficient of 0.9998.

**Specificity**

The HPLC chromatogram showed that there was no interference of any of the formulae ingredients at the retention time of piroxicam at 4.7 minutes (Fig. 1.2a and 1.2b).

**Precision and accuracy**

Table 1.1 shows that the intra-day accuracy values are ranged between 85.06-101.94% with a precision of 1.70-6.15%. Similarly, Table 1.2 shows that the inter-day accuracy ranged between 86.50-107.76% with a precision of 0.41-5.88%. The precision and accuracy of all concentration values were less than ±15%. The results showed that the method is reproducible and precise within and between the analytical runs during the same day and on different day.

**Table 1.1:** Intra-day accuracy and precision results of piroxicam. All data are presented as mean±SD, (N=6).

| Nominal concentration (μg/ml) | Actual concentration (μg/ml) | Accuracy (%) | Precision (%) |
|---|---|---|---|
| 0.0625 | 0.0718 (0.0012) | 85.06 | 1.70 |
| 0.1250 | 0.1326 (0.0082) | 93.91 | 6.15 |
| 0.2500 | 0.2475 (0.0134) | 100.99 | 5.43 |
| 0.5000 | 0.4903 (0.0086) | 101.94 | 1.75 |
| 1.0000 | 0.9865 (0.0272) | 101.35 | 2.75 |
| 2.0000 | 2.0087 (0.0576) | 99.57 | 2.87 |

*Values in parenthesis are the standard deviations*

**Limit of detection and limit of quantification**

Based on the peak height of 3 times the noise level, the limit of detection was 0.035 μg/ml and based on the peak height of 5 times the noise level, limit of quantification was 0.0625 μg/ml.

**Table 1.2: Inter-day accuracy and precision results of piroxicam. All data are presented as mean±SD, (N=6).**

| Nominal concentration (µg/ml) | Actual concentration (µg/ml) | Accuracy (% ) | Precision (%) |
|---|---|---|---|
| 0.0625 | 0.0709 (0.0021) | 86.51 | 2.93 |
| 0.1250 | 0.1390 (0.0082) | 88.78 | 5.88 |
| 0.2500 | 0.2306 (0.0026) | 107.76 | 1.11 |
| 0.5000 | 0.4878 (0.0020) | 102.43 | 0.41 |
| 1.0000 | 0.9955 (0.0282) | 100.45 | 2.84 |
| 2.0000 | 2.0067 (0.0132) | 99.67 | 0.66 |

*Values in parenthesis are the standard deviations*

## Acknowledgements

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