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A New Isocratic HPLC Method for Simultaneous Determination of Paracetamol and Flurbiprofen in Pharmaceutical Preparations

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Abstract
An isocratic HPLC method has been developed for simultaneous determination of paracetamol-acetaminophen (PAR) and flurbiprofen (FLU) in pharmaceutical preparations. The method was based on separation by using a mobile plase acetonitrile– 0.1M sodium acetate solution (pH:5.00) (60:40,v/v) and reversed-phase C_{18} column. The mobil plase flow rate was 0.8mL/min with UV detection at 247nm. The linearity ranges were 0.1-1.5 µg/mL for PAR and FLU. The limits of detection and quantification were found to be 16.33 ng/mL and 54.45 ng/mL for PAR and 15.25 ng/mL and 50.82 ng/mL for FLU, respectively. The combination of these two drugs has not commercially avaible but patented as combined preparation. The proposed method was successfully validated and applied to the determination in pharmaceutical preparations. The developed and validated method is simple, sensitive and reproducible and can be used safely routine simultaneous analysis of PAR and FLU in pharmaceutical preparations in the future.

Keywords: Paracetamol; Acetaminophe; Flurbiprofen; HPLC; Pharmaceutical preparations.

1. Introduction

Paracetamol (PAR), *N*-acetyl-*p*-aminophenol , acetominophen (Figure 1) is an analgesic and antipyretic drug for relief of pain and fever reduction [1].

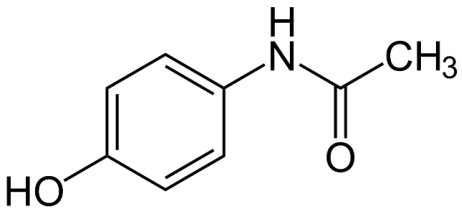

Figure 1. Chemical structure of PAR

Flurbiprofen, (±)-2-(2-fluoro-4-biphenyly) propionic acid (Figure 2) is an important non-steroidal, anti-inflammatory drug (NSAID). It is usually recommended to the patients with rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and gout [2]. Various chromatographic methods were described for determination of PAR by HPLC with UV [3-12], and LC-MS/MS analysis [13-15], spectrophotometric [16]. Some electrochemical methods were also carried out by voltammetry [17, 18].

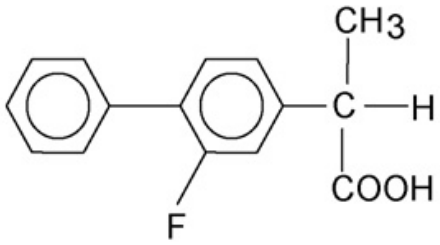

Figure 2. Chemical structure of FLU

Several LC methods using UV detection [19-26], LC-MS/MS [27, 28] and fluorescence detection [29] techniques have been reported for the estimation of FLU either alone or together with their metabolites in plasma and tablet form.
Although there is no any commercial preparation of PAR and FLU simultaneously, a patent has been developed for some preparations containing each other [30]. For this purpose, an isocratic LC method for simultaneous determination of PAR and FLU in tablet form has been developed and validated in this study.

2. Experimental

2.1. Chemicals and Reagents

PAR and FLU was obtained from (Atabay Kimya Sanayi ve Ticaret A.Ş.and FDC Limited). Methanol which is HPLC grade was purchased from Sigma-Aldrich. Acetonitrile which is HPLC grade purchased from VWR. All other chemicals were analytical grade. HPLC grade solvents were used. Purified water for the HPLC was prepared through a Milli-Q® Integral Ultrapure Water Purification System (Merck Millipore).

2.2. Pharmaceutical Formulation

Fixed-dose combination tablets containing 150 mg PAR, 100 mg FLU for active ingredient and 75 mg lactose, 50 mg corn starch, 4 mg magnesium stearate in each tablet were prepared. Ten tablets of the preparation were weighted and finely powdered.

2.3. Chromatographic Conditions

The HPLC system of Agilent Infinity 1260 Series HPLC-UV were used for analysis of PAR and FLU. The data acquisition and analysis were performed using the Chem32 software (Agilent Technologies).
Chromatographic separation was carried out using a reversed phase Intersil ODS, 5 µm, 250x4.6 mm column at 25 ^{0}C temperature. The mobile phase was consist of (acetonitrile: 0.1M sodium acetate pH:5) (60:40) (v/v). Flow rate of mobile phase was 0.8 mL/min. The detector was set 247 nm. The injection volume was 20 µL.

2.4. Preparation of Standard Solutions

Stock solutions of PAR (1.0 mg/mL) and FLU (1.0 mg/mL) were prepared and diluted to 10µg/mL with acetonitrile. All solutions were stored at 4ºC. The five calibration standards were prepared with acetonitrile between the concentration ranges of 0.1-1.5 µg/mL. The quality control (QC) samples were prepared at concentrations of 100, 500, 1000, and 1500 ng/mL. All calibration standards and QC samples were stored at 4ºC.

2.5. Method Validation

The method was validated according to ICH guidelines for linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ), robustness, and system suitability.
The linearity was evaluated by analyzing different concentrations of the standard solutions of PAR and FLU. The calibration curves were constructed over the concentration range of 0.1 – 0.3 – 0.6 – 0.75 - 1.5 µg mL−1 for PAR and 0.1- 0.2- 0.4- 0.75- 1.5µg mL−1 for FLU, and the linearity was evaluated by plotting the peak area against the concentration. The linear regression equation was computed.
LOD and LOQ values of the method were evaluated using the following equations:

LOD = 3 x σ /S
LOQ = 10 x σ /S

where σ is the standard deviation of the response and "S" is the slope of the calibration curve.

2.6. Analysis of Dosage Form

Ten tablets were powdered and equal to one tablet was weighed. Then it was diluted to volume with acetonitrile in a 100 mL flask (6 replicates). Further dilution was carried out by transferring appropriate amounts for final concentrations of 0.6µg/mL PAR and 0.4µg/mL FLU.

3. Results and Discussion

Chromatographic separation of analytes were optimized to provide acceptable resolution, good peak shape and intensity of the response. Mobile phase composition was changed systematically to establish chromatographic conditions giving an acceptable resolution. A typical LC chromatogram of standard mixture containing PAR and FLU is given in Figure 3.

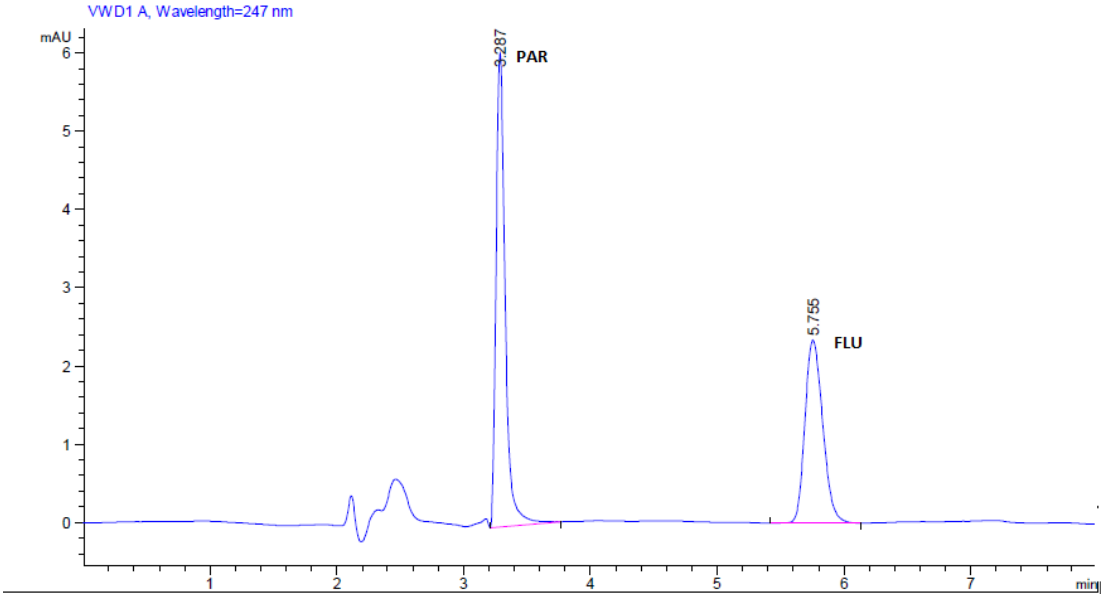

Figure 3. LC chromatogram of standard mixture containing PAR (0.3 µg/mL) and FLU (0.2 µg/mL)

The selectivity of method was assessed by analyzing inactive ingredients of pharmaceutical preparations. Chromatograms were compared for any interference from the matrix or any of the assay reagents.
The linearity ranges were 0.1-1.5 µg/mL for both of PAR and FLU. The calibration curves were prepared from five calibration samples within the range of 0.1-1.5 µg/mL. The acceptance criteria of back calculated standard concentration was 15% deviation from nominal value except the LOQ (for LOQ 20 % deviation was applied). The standart calibration curves were linear over the concentration range from 1.5 µg/mL with mean r^2=0.9994 for PAR and r^2=1.0000 for FLU. LOD and LOQ values were found to be 16.33 ng/mL and 54.45 ng/mL for PAR and 15.25 ng/mL and 50.82 ng/mL for FLU, respectively (Table 1).

Table 1. Linearity values of PAR and FLU

Drug	Concentration Range ($ngmL^{-1}$)	Regression Equation	Correlation Coefficient (r^2)	LOD ($ngmL^{-1}$)	LOQ ($ngmL^{-1}$)
PAR	100 - 1500	y=0,110118x – 1,58599	0,9994	16,33	54,45
FLU	100 - 1500	y=0,116354x – 1,07823	1,0000	15,25	50,82

Intra batch accuracy and precision were determined by analysis of six replicates of 5 concentrations including low, medium and high concentration QC samples. Inter-batch accuracy and precision were

determined by the analysis of these QC samples on three separate states. The overall precision of the method was expressed as percentage of coefficient of variation and the accuracy of the method was expressed in terms of relative errors. Table 2 gives a summary of the precision. Recovery values were found 101.13 % ± 0.41 for PAR and 98.17% ± 0.77 for FLU in pharmaceutical preparation (Table 3).

Table 2. Precision studies of PAR and FLU

Drug	Concentration ($ngmL^{-1}$)	Interday Determination (n=6)			Interday Determination (n=6)		
		Peak Area (mAu*S)	Concentration Measured ($ngmL^{-1}$)	%RSD	Peak Area (mAu*S)	Concentration Measured ($ngmL^{-1}$)	%RSD
PAR	500	53,9496	504,3290	0,9351	53,5319	501,5600	0,7516
	1000	109,3039	1007,0110	0,5681	108,2281	1001,6890	1,1645
	1500	164,4164	1507,5060	0,3615	163,8811	1502,6343	0,3615
FLU	500	58,02584	507,9677	0,9814	57,4288	502,8361	1,1286
	1000	116,0660	1006,7916	0,4954	115,4786	1001,7432	1,0371
	1500	174,6009	1509,8600	0,50245	173,9653	1504,4049	1,1461

Table 3. Accuracy studies of PAR and FLU

Drug	Percent of Nominal (level)	Amount Added ($ngmL^{-1}$)	Amount Recoverd (($ngmL^{-1}$) ± RSD%))	% Recovery
PAR	50%	300	300,7962 ± 1,3389	100,26
	100%	600	604,9395 ± 0,7354	100,82
	125%	750	745,5867 ± 0,8864	99,41
FLU	50%	300	301,1910 ± 0,3643	100,39
	100%	600	611,8420 ± 0,9112	101,97
	125%	750	755,8400 ± 0,6784	100,77

4. Conclusion

A new isocratic HPLC method has been developed and validated for simultaneous determination of PAR and FLU in pharmaceutical preparations in the first time. This method; simple, accurate, precise and selective can be used for the quality control analysis of PAR and FLU in pharmaceutical preparations in the future.

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