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HPLC Method for the Simultaneous Determination of Paracetamol, Aceclofenac and Tizanidine Hydrochloride

VANITA SOMASEKHAR[1,*] and DANNANA GOWRISANKAR[2]

[1]K.L.E. University's College of Pharmacy, Rajajinagar, Bangalore-560 010, India
[2]College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530 003, India

*Corresponding author: E-mail: vanitasom@gmail.com

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A novel and simple HPLC method was developed and validated for the simultaneous determination of paracetamol, aceclofenac and tizanidine hydrochloride in pharmaceutical preparations. The optimum separation for these analytes was achieved in less than 11 min with a Phenomenex Microsorb-MV 100 C_{18} column and a mobile phase containing methanol, water, terahydrofuran and orthophosphoric acid in the ratio 560:400:40:1. Detection wavelength was set at 290 nm. The method was suitably validated with respect to specificity, linearity, limit of detection and quantification, accuracy, precision and robustness and was successfully applied to the simultaneous determination of paracetamol, aceclofenac and tizanidine hydrochloride in pharmaceutical preparations.

Key Words: Paracetamol, Aceclofenac, Tizanidine hydrochloride, HPLC, Validation.

INTRODUCTION

Paracetamol is chemically N-(4-hydroxyphenyl) acetamide and used mainly as antipyretic. Aceclofenac is 2[(2,6-dichlorophenyl)amino]benzoic acid carboxymethyl ester with potent analgesic and antiinflammatory properties with improved gastric tolerance. Tizanidine hydrochloride, is a short-acting drug for the management of spasticity.

Literature survey reveals several methods for the determination of paracetamol alone or in combination with other drugs in pharmaceutical preparations or in biological fluids in recent years, including liquid chromatography (LC)[1-3], liquid chromatography-tandem mass spectrometry (LC/MS-MS)[4-7], high performance thin layer chromatography (HPTLC)[8-11], capillary electrophoresis[12] and spectrophotometry[13-17].

Several methods have also been described in recent years for the determination of aceclofenac either alone or in combination with various drugs, such as liquid chromatography-tandem mass spectrometry (LC/MS-MS)[18], liquid chromatography (LC)[19-22] and spectrophotometry[22,23].

Similarly, several methods have been reported for the determination of tizanidine hydrochloride either alone or in combination with various drugs, such as liquid chromatography (LC)[24-28], HPTLC[27,29] and spectrophotometry[30-32].

To our best of knowledge only two methods have been published recently for the determination of these three drugs from combined dosage form, based on LC[33,34].

Market is flooded with combination of drugs in various dosage forms. The multi-component formulations have gained a lot of importance nowadays due to greater patient acceptability, increased potency, multiple action, fewer side effects and quicker relief[35]. The aim of this study was to demonstrate method development and validation strategies of HPLC for the analysis of paracetamol, aceclofenac and tizanidine hydrochloride. For this purpose, the influence of buffer type, buffer concentration and organic modifier was systematically investigated and the method validation studies were performed. The validated method was successfully applied to the pharmaceutical preparations. Although there is a large difference in the label claims of paracetamol, aceclofenac and tizanidine hydrochloride per tablet, simultaneous analysis of these three drugs has been achieved in the same sample preparation.

EXPERIMENTAL

All HPLC experiments were performed on a Shimadzu (Kyoto, Japan) system consisting of LC-10AT pump, a SPD-10A UV detection and an automatic sample injector, using a Phenomenex Microsorb-MV 100 C_{18}, 5 µm, 250 mm × 4.6 mm i.d. column at ambient temperature and eluted with mobile phase at the flow rate of 1.0 mL/min. All experiments were conducted with ultra pure water obtained from Milli-Q academic system (Millipore Pvt. Ltd., Bangalore, India). All solutions were degassed by ultrasonication.

Paracetamol, aceclofenac and tizanidine hydrochloride reference standards were kindly supplied by Anglo-French

Drug Industries, Bangalore, India. The tablets containing paracetamol, aceclofenac and tizanidine hydrochloride were marketed samples purchased from a local pharmacy. Methanol, terahydrofuran and orthophosphoric acid were of HPLC grade. All other chemicals were analytical reagent grade. Mobile phase was filtered through 0.45 µm nylon filter.

Standard and sample solutions

Standard solutions: Standard stock solution was prepared by dissolving 500 mg of paracetamol, 50 mg of aceclofenac and 100 mg of tizanidine hydrochloride in the diluting solution (methanol, potassium phosphate 0.05 M and glacial acetic acid in the ratio 700:300:5) and making up to 100 mL with the same. This solution was kept at +4 ºC. Standard solution was prepared by diluting 5 mL of standard stock solution to 50 mL with diluting solution.

Mobile phase: Optimized mobile phase was prepared by dissolving 1.776 g of docusate sodium in 1000 mL mixture of methanol, water, tetrahydrofuran and orthophosphoric acid in the ratio 560:400:40:1.

Sample preparation: Twenty tablets were weighed and finely powdered in a mortar. 1 g of sample powder was accurately weighed and transferred to a 50 mL volumetric flask. 30 mL of diluting solution was added, sonicated for 10 min and diluted to the mark with the diluting solution.

Sample solution was filtered through a 0.45 µ syringe filter and degassed with ultrasonic bath for 10 min before injection.

RESULTS AND DISCUSSION

Method development and optimization: Manipulation of mobile phase composition is a key factor for optimizing the separation and elution of the analytes in HPLC. Taking into consideration of different parameters (migration time, resolution, peak shape, *etc*.) best results were obtained by the addition of two organic modifiers, methanol and tetrahydrofuran and by the addition of orthophosphoric acid for pH adjustment. But the elution behaviour exhibited by aceclofenac and tizanidine hydrochloride were almost the same. Hence docusate sodium was added which delayed the elution of aceclofenac more than that of tizanidine hydrochloride, thereby separating the two peaks.

In order to increase the sensitivity of the method, detection wavelength was selected as 290 nm at which tizanidine hydrochloride has maximum absorption.

Method validation: Validation of the proposed method was performed with respect to specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), range, accuracy, precision and robustness according to the ICH guidelines[36].

Specificity: Specificity, described as the ability of a method to discriminate the analyte from all potential interfering substances was evaluated by preparing an analytical placebo and it was confirmed that the signals measured was caused only by the analytes. A solution of an analytical placebo (containing all ingredients of the formulation except the analyte) was prepared according to the sample preparation procedure and injected. To identify the interference by these excipients, a mixture of the inactive ingredients (placebo), before and after being spiked with standard (Fig. 1) and the commercial pharmaceutical preparations including paracetamol, aceclofenac and tizanidine hydrochloride were analyzed by the proposed method. The representative chromatograms showed no other peaks, which confirm the specificity of the method.

Linearity: Under the optimum analysis conditions, linearity was studied simultaneously in the concentration range of 0.5-2.5 mg mL^{-1} for paracetamol, 0.05-0.25 mg mL^{-1} for aceclofenac and 0.1-0.5 mg mL^{-1} for tizanidine hydrochloride. The linearity curves were defined by the following equations: y = 7E + 06x, r = 0.9948 for paracetamol, y = 7E + 06x, r = 0.9998 for aceclofenac and y = 3E + 06x, r = 0.9962 for tizanidine hydrochloride, where y is the area and x is the concentration expressed in mg mL^{-1} (n = 3). The equation of linear regression and statistical data for paracetamol, aceclofenac and tizanidine hydrochloride are presented in Table-1. The linearity of the calibration curve for each drug was validated by the high value of the correlation coefficient.

LOD and LOQ: The limit of detection is defined as LOD = 3.3 σ/s, where σ denotes standard deviation of response and s denotes slope.

The limits of detection were determined as 0.57 mg mL^{-1} for paracetamol, 0.11 mg mL^{-1} for aceclofenac and 0.03 mg mL^{-1} for tizanidine hydrochloride. The limit of quantification is defined as LOQ = 10 σ/s.

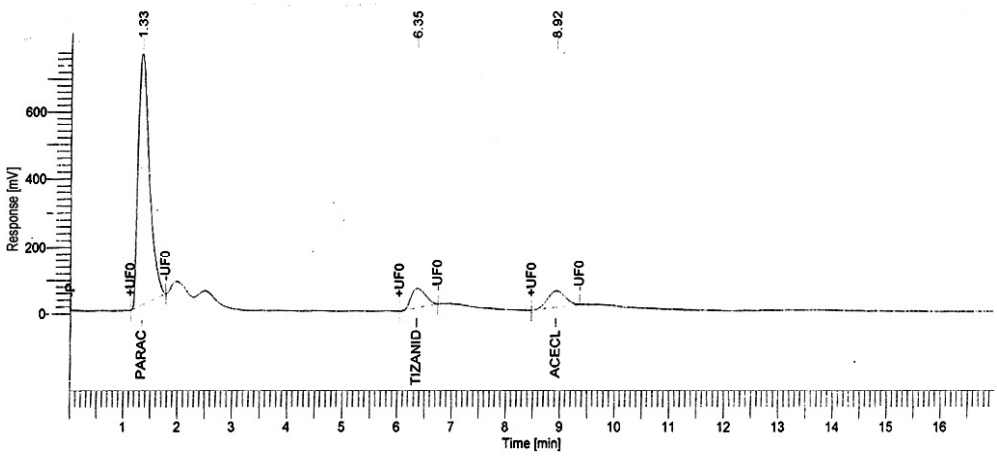

Fig. 1. Representative chromatogram obtained from blank spiked with standard solution

TABLE-1
STATISTICAL DATA OF CALIBRATION CURVES OF PARACETAMOL, ACECLOFENAC AND TIZANIDINE HYDROCHLORIDE

Parameters	Paracetamol	Aceclofenac	Tizanidine hydrochloride
Linearity (mg mL^{-1})	0.5-2.5	0.05-0.25	0.1-0.5
Regression equation	y = 7E + 06x	y = 7E + 06x	y = 3E + 06x
Correlation coefficient (r)	0.9948	0.9998	0.9962
Limit of detection (LOD) (mg mL^{-1})	0.57	0.11	0.03
Limit of quantitation (LOQ) (mg mL^{-1})	1.74	0.33	0.10

The limits of quantification were determined as 1.74 mg mL^{-1} for paracetamol, 0.33 mg mL^{-1} for aceclofenac and 0.10 mg mL^{-1} for tizanidine hydrochloride.

Precision: The assay was investigated with respect to system suitability test, method precision and intermediate precision. The system suitability test and method precision were carried out to monitor repeatability and reproducibility.

In order to measure repeatability of the system (system suitability test), six consecutive injections were made with the standard solutions and the results were evaluated by considering peak area values of paracetamol, aceclofenac and tizanidine hydrochloride. The precision values with their RSD are shown in Table-2. The results in Table-2 indicate that the RSD (%) is less than 2 % for paracetamol, aceclofenac and tizanidine hydrochloride.

Six different sample weights of paracetamol, aceclofenac and tizanidine hydrochloride were analyzed in three independent series in the same day (intra-day precision) and three consecutive days (inter-day precision), within each series every sample was injected three times. The RSD values of intra- and inter-day studies (Table-3) varied from 0.23-2.25 % showing that the intermediate precision of the method was satisfactory.

Accuracy and recovery studies: The accuracy of a method is expressed as the closeness of agreement between the value found and the value that is accepted as a reference value. It is determined by calculating the per cent difference (bias %) between the measured mean contents and the corresponding nominal contents[37]. Table-3 shows the results obtained for intra- and inter-day accuracy.

The accuracy of the proposed method was also tested by recovery experiments. Recovery experiments were performed by taking different sample weights and spiking with paracetamol, aceclofenac and tizanidine hydrochloride at three different concentration levels (50 mg paracetamol/15 mg aceclofenac/22 mg tizanidine hydrochloride; 100 mg paracetamol /30 mg, aceclofenac/44 mg tizanidine hydrochloride; 150 mg paracetamol/45 mg aceclofenac/66 mg tizanidine hydrochloride). Three samples were prepared for each recovery level. Samples were treated as described in the procedure for sample preparations.

TABLE-2
REPEATABILITY OF SYSTEM (n = 6)

	Peak areas		
	Paracetamol	Aceclofenac	Tizanidine hydrochloride
Mean ± standard error	4200291 ± 27031.7	413526.1 ± 1134.6	418806.6 ± 1776.6
Standard deviation	66213.8	2779.3	4351.7
Relative standard deviation (%)	1.58	0.67	1.04

TABLE-3
ACCURACY AND INTERMEDIATE PRECISION DATA FOR PARACETAMOL, ACECLOFENAC AND TIZANIDINE HYDROCHLORIDE

	Intra-day			Inter-day			
Taken (mg/tablet)	Found[a] (mg/tablet)	Precision[b] (RSD (%))	Accuracy[c] (bias %)	Taken (mg/tablet)	Found (mg/tablet)	Precision (RSD (%))	Accuracy (bias %)
Paracetamol 500	501.6 ± 0.49	0.23	0.32	500	497.2 ± 2.49	1.22	0.56
Aceclofenac 100	100.7 ± 0.41	1.00	0.70	100	100.4 ± 0.57	1.39	0.4
Tizanidine hydrochloride 2	2.0 ± 0.02	2.25	0	2	2.0 ± 0.01	1.44	0

[a]Mean ± standard error. [b]RSD (%): Relative standard deviation. [c]bias (%): [(found – taken)/taken] × 100.

TABLE-4
RECOVERY DATA FOR PARACETAMOL (P), ACECLOFENAC (A) AND TIZANIDINE HYDROCHLORIDE (T) (n = 6)

Dosage (P/A/T) (mg)	Amount added (mg)	Amount found ± SD (mg)	Recovery (%)	RSD (%)
500/100/2				
Paracetamol	50	550.6 ± 0.21	100.1	0.06
Aceclofenac	15	114.9 ±0.40	99.9	0.35
Tizanidine hydrochloride	22	23.9 ± 0.12	99.9	0.46
500/100/2				
Paracetamol	100	597.4 ± 1.56	99.6	0.31
Aceclofenac	30	128.9 ± 0.65	99.1	0.50
Tizanidine hydrochloride	44	46.8 ± 0.40	101.7	0.91
500/100/2				
Paracetamol	150	645.4 ± 0.21	99.3	0.00
Aceclofenac	45	144.3 ± 0.91	99.5	0.66
Tizanidine hydrochloride	66	67.6 ± 0.38	99.4	0.55

TABLE-6
DATA FROM THE ANALYSIS OF COMMERCIAL TABLETS (n = 3)

Ingredient	Labeled claim (mg)	Found ± SD (mg)	Reference method	RSD (%)
Paracetamol	500	498.6 ± 2.39 t = 0.34; F = 3.098	499.13 ± 1.36	0.48
Aceclofenac	100	99.97 ± 0.42 t = 0.22; F = 2.558	99.87 ± 0.67	0.42
Tizanidine hydrochloride	2	1.93 ± 0.057 t = 1.15E - 18; F = 7	1.93 ± 0.15	2.95

The results obtained are shown in Table-4, from which it is clear that both the recoveries and repeatabilities are excellent.

Robustness: Robustness relates to the capacity of the method to remain unaffected by small but deliberate variations introduced into the method parameters. The detection wavelength was varied from 290-300 nm and the recoveries obtained for paracetamol, aceclofenac and tizanidine hydrochloride were not different compared to the actual contents (Table-5).

TABLE-5
ROBUSTNESS DATA

Paracetamol		Aceclofenac		Tizanidine hydrochloride	
Amount found (mg)	Amount taken (mg)	Amount found (mg)	Amount taken (mg)	Amount found (mg)	Amount taken (mg)
500.8	500	99.9	100	1.94	2.0

Analysis of pharmaceutical formulations: In order to evaluate the applicability and reliability of the proposed methodology, it was applied to the simultaneous determination of paracetamol, aceclofenac and tizanidine hydrochloride in tablets. Satisfactory results were obtained for each compound and were found to be in agreement with label claims (Table-6). The results obtained by the proposed method were compared with those obtained with the reference method and were found to be equally accurate.

Conclusion

A simple, rapid and sensitive method for the simultaneous estimation of paracetamol, aceclofenac and tizanidine hydrochloride in pharmaceutical preparations has been developed and validated. The major advantage of this method is the quick sample analysis without prior separation or purification. Sample preparation procedure was simple with a short chromatographic time making the method suitable for processing multiple samples in a limited period of time. Hence, it can be concluded that the proposed method is useful and suitable for routine quality control tests such as content uniformity of commercial formulations of paracetamol, aceclofenac and tizanidine hydrochloride.

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