Eurasian Journal of Analytical Chemistry

Eurasian J Anal Chem 10(3): 163-179, 2015

Development and Validation of Reversed Phase High Performance Liquid Chromatographic Method for Simultaneous Estimation of Paracetamol, Caffeine and Carisoprodol in Tablet Formulation

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Received: *13/08/2015****; Accepted:*** *10/09/2015*

Abstract

Combined dose tablet formulation containing Paracetamol, Caffeine and Carisoprodol is used for the treatment of low back pain, post traumatic muscle spasm, sprains, strains and tenosynovitis. In this study a simple, specific, precise and accurate reverse phase high performance liquid chromatographic (RP-HPLC) methods has been developed for simultaneous estimation of paracetamol (PAR), caffeine (CAF) and carisoprodol (CAR) in tablet dosage form. In the proposed chromatographic method separation was achieved by HiQ silC-18HS column (250 mm× 4.6 mm), with mobile phase containing Acetonitrile: Buffer (0.1mol L^{-1} Orthophosphoric Acid) (30:70 v/v) and the pH of the Buffer was adjusted to 3.1 by triethylamine. The flow rate was 1.0 ml min^{-1} and effluent was monitored at 232.2 nm. The retention time of CAF, PAR and CAR were 2.804 min, 4.815 min and 6.718 min respectively. The linearity for PAR, CAF and CAR were in the range of 5-25 µg mL^{-1}, 5-25 µg mL^{-1} and 10-50 µg mL^{-1} respectively. The recoveries of PAR, CAF and CAR were found in the range of 99.05-99.78 %, 98.47-99.90 % and 98.92-99.56 % respectively. The proposed methods were validated as per International Conference on Harmonisation (ICH) guidelines by means of different parameters likes linearity, precision, accuracy, and limit of detection, limit of quantitation, range and selectivity, robustness ruggedness, solution stability as per ICH guidelines and successfully applied to the estimation of PAR, CAF and CAR in the tablet dosage form.

Keywords:

Paracetamol, Caffeine, Carisoprodol, RP-HPLC, Simultaneous estimation

1. Introduction

PAR is a chemically N-acetyl-p-aminophenol (Fig. 1). PAR is a non-opioid, non-salicylate analgesic with an unclear mechanism of action [1]. PAR is official in IP, BP and USP. Literature survey reveals various UV and chromatographic methods are available for estimation of PAR in single and combined dosage forms. Literature survey also reveals LC-MS, GC-MS, IR and HPTLC methods are reported for estimation of PAR with other drugs in combination [2-14].

CAF chemically is 1, 3, 7-Trimethyl-1H-purine-2, 6 (3H, 7H)-Dione (Fig. 3). It is a central nervous system stimulant. It acts by inhibition of cyclic nucleotide phosphodiesterases, antagonism of adenosine receptors, and modulation of intracellular calcium handling [15].

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ISSN: 1306-3057

Caffeine is official in IP, BP, and USP. Literature survey also reveals that various analytical methods are reported for estimation of CAF with other drugs in combination [16-21].

CAR chemically is (*RS*)-2-{[(aminocarbonyl) oxy] methyl}-2-methylpentyl isopropylcarbamate (Fig. 2). Carisoprodol is a CNS depressant which has sedative and skeletal muscle relaxant effects. The precise mechanism of action of the drug is not known. Carisoprodol does not appear to directly relax tense skeletal muscles in man. In animals, Carisoprodol produces muscle relaxation by blocking interneuronal activity in the descending reticular formation and spinal cord [22]. It is official in EP and USP. Literature survey also reveals UV, MS-MS, LC-MS-MS, and GC-MS methods are reported for estimation of CAR with other drugs in combination [23-29].

The combination of CAR, CAF and PAR (Carisoma compound tablet) is used for treatment of low back pain, post traumatic muscle spasm, sprains, strains and tenosynovitis. According to literature survey, there was not any developed analytical method which has been reported for simultaneous estimation of CAR, PAR and CAF in combined dosage form. So an attempt was being made to develop simple, accurate, precise, economical and reproducible chromatographic method for simultaneous estimation of CAR, PAR and CAF in tablet dosage form. The developed method was validated in accordance with ICH guideline [30,31] and successfully employed in the assay of CAR, PAR and CAF in combined tablet dosage form.

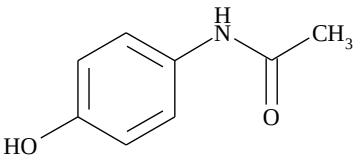

Fig. 1. Paracetamol

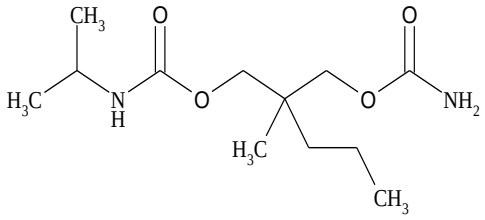

Fig. 2. Carisoprodol

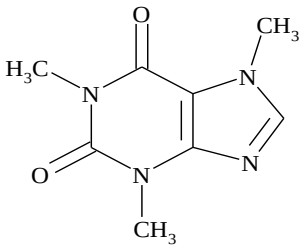

Fig. 3. Caffeine

2. Experimental

2.1 Materials and Reagents

The standard PAR and CAF were obtained from Wockhardt Ltd., Aurangabad, India. CAR was obtained from Watson (Actavis) Pharmaceutical Pvt. Ltd, Goa, India. Deionised distilled water (DIW) used was obtained from Loba Chemie Mumbai, India. HPLC grade Acetonitrile and methanol were obtained from Merck Ltd., India. Buffering agent Orthophosphoric Acid and triethylamine were procured from Fisher scientific, Mumbai. India. Marketed formulations containing PAR, CAF and CAR was procured from the local pharmacy market.

2.2 Chromatographic system and conditions

Liquid chromatography was performed on JASCO Isocratic HPLC system model LC-NET II/ADC (JASCO Corporation, Japan). The system built with UV-2070 as UV-VIS detector and HiQ sil C18HS (4.6 × 250 mm, 5µm) column with a 20 µL manual sample injector. The HPLC system was equipped with Chrom-NAV software for data processing.

All compounds were eluted off the column with a mobile phase consisting of Acetonitrile: Buffer (0.1 mol L^{-1} orthophosphoric acid, 30:70 v/v PH 3.1 adjusted with triethylamine) at a flow rate of 1.0 ml/min in isocratic mode. The mobile phase was filtered through a 0.45 µm nylon filter and then ultrasonicated for 30 min. The injection volume was 20 µL and the eluent was detected at 232.2 nm, which was selected as wavelength for further

analysis. The retention time of PAR, CAF and CAR were around 4.81, 2.80 and 6.71 min, respectively, and the total run was 10 min (Table 2). The method was validated in accordance with the ICH guidelines for validation of analytical procedures [30,31].

2.3 Assay of tablet formulation

Twenty tablets were taken, containing 350 mg of PAR, 32 mg of CAF and 175 mg of CAR. The tablets were crushed to fine powder and a precisely weighed portion of the powder equivalent to 3.5 mg PAR, 0.32 mg CAF and 1.75 mg CAR was weighed accurately, and then transferred to 100 mL dried volumetric flask. Sufficient amount of mobile phase was added to dissolve the content and resulting solution was shaken for 20 min. The volume was made up to 100 ml with the mobile phase and then filtered through membrane filter and degassed in sonicator. From this solution appropriate dilutions of PAR, CAF and CAR were made to get the final concentrations. After that sample was injected into the HPLC system to get chromatogram. The chromatogram obtained is shown in Fig. 9 and the area obtained in each chromatogram of five replicates was correlated with regression equation and the amount found was calculated, which was within the limit of label claim as mentioned in Table 1.

Table 1. Analysis of Tablet Formulation

Drug	Label Claim (mg/tab)	Peak area (μv/sec)	% of Label claim determine	Mean %	SD*	RSD*
PAR	350	587683	99.49	99.89	0.2828	0.2837
CAF	32	104555	99.01	100.09	0.2404	0.2406
CAR	175	1094213	99.45	99.65	0.1414	0.1420

* indicates avreage of five determination

Table 2. Optimal chromatographic conditions of tablet formulation

Aspect	Description
Mobile phase	Acetonitrile: Buffer (0.1M Orthophosphoric Acid, 30:70 v/v PH 3.1 adjusted with triethylamine)
HPLC Column	HiQ sil C18HS (4.6 × 250 mm, 5μm)
Flow rate	1.0 ml/min
Injection volume	20 μl
Retention time	for PAR, CAF and CAR ware 4.81, 2.80 and 6.71 min
Runtime	10 min

2.4 Method validation

2.4.1 Specificity and selectivity

The Specificity and selectivity parameters were determined by comparing the chromatograms of the PAR, CAF and CAR standard, tablet formulation and mobile phase as a solvent.

2.4.2 Linearity

The linearity of an analytical procedure is its ability within a given range to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample [30,31]. The linearity is the relationship between peak area and the concentration was determined by analyzing over the concentration range of 5-25 μg mL^{-1} for PAR, 5-25 μg mL^{-1} for CAF and 10-50 μg mL^{-1} for CAR.

2.4.3 Accuracy

To check the degree of accuracy of the method, recovery studies were performed in triplicate by the standard addition method at 50%, 100% and 150%. Known amounts of standard PAR, CAF and CAR were added to the pre-analyzed samples and were subjected to the proposed HPLC method.

2.4.4 Precision

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). The repeatability was calculated as the relative standard deviation with three replications and three different concentrations during the same day. Intermediate precision was studied by comparing the assays on two different days.

2.4.5 Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Limit of detection can be calculated using the following equation as per ICH guidelines [30,31].

$$LOD = 3.3 \times N/S$$

Where, N is the standard deviation of the peak area of the drug and S is the slope of the corresponding calibration curve.

2.4.6 Limit of Quantification (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. Limit of quantification can be calculated using the following equation as per ICH guidelines [30,31].

$$LOQ = 10 \times N/S$$

Where, N is the standard deviation of the peak area of the drug and S is the slope of the corresponding calibration curve.

2.5 Method optimization

Four parameters were optimized to get better separation. These parameters were mobile phase, flow rate, wavelength and injection volume.

2.6 Selection of analytical wavelength

By appropriate dilution of each standard stock solution in the mobile phase, various concentrations of PAR, CAF and CAR were prepared separately. Each solution was scanned in between the range of 200-400 nm and their overlain spectrum was taken. The isobestic point was observed at 232.2 nm in the overlain spectra of PAR, CAF and CAR. The wavelength selected for the HPLC analysis was 232.2 nm to which these three drugs showed significant absorbance and very good resolution. The overlain UV spectrum of PAR, CAF and CAR in the mobile phase is shown in Fig. 4.

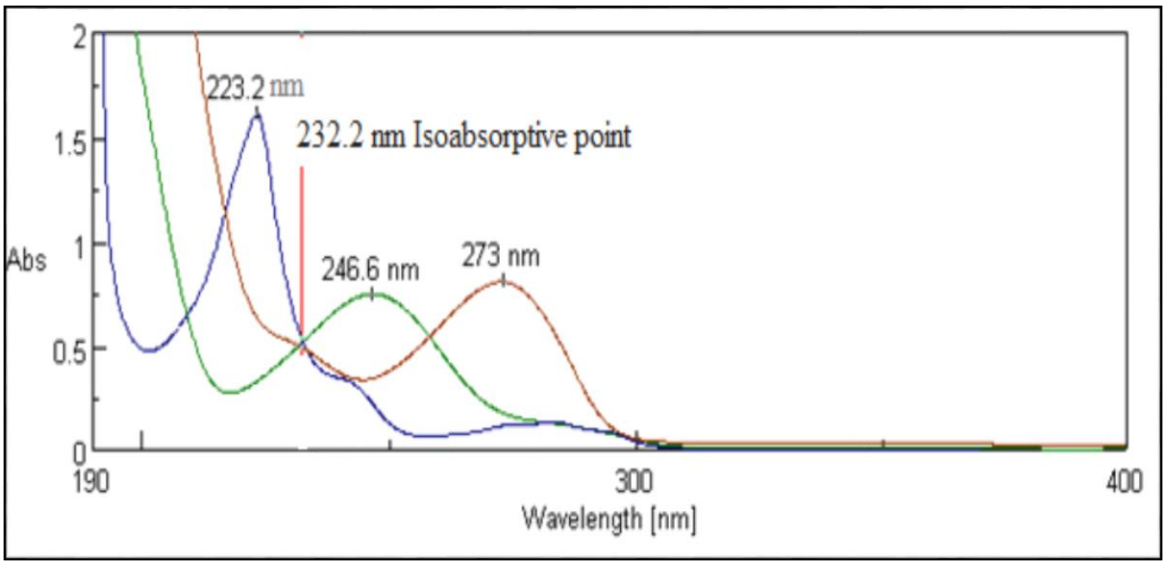

Fig. 4. Overlain Spectra of PAR, CAF and CAR (232.2 nm) in mobile phase

3. Results and discussion

3.1. Analytical method development

The optimization of mobile phase, flow rate, wavelength and injection volume is considered very vital to achieve good separation and peak area. In the proposed method of estimating these four parameters were optimized separately for PAR, CAF and CAR then optimized for in combination. In this investigation, we observed no significant difference in the results obtained with the mobile phase Acetonitrile: Buffer (30:70 v/v, PH 3.1). The mobile phase made up of 100% acetonitrile produced too late peak with an area lower than last mobile phase, maybe this is attributed buffer effect.

In case of these three mobile phases (acetonitrile/buffer, 50:50; acetonitrile/buffer, 60:40; acetonitrile/methanol/buffer, 50:40:10) less resolution and late elution peak were obtained. Different trials (acetonitrile: 0.1 mol L^{-1} orthophosphoric acid buffer; 30:70 v/v) were conducted at varying of pH range (2-5) of 0.1 mol L^{-1} orthophosphoric acid buffer with satisfactory results, but non-symmetrical peak and smaller number of theoretical plates were observed. The mobile phase chosen for analytical method validation was Acetonitrile: Buffer (30:70 v/v,) at PH 3.1, presented a mobile phase holdup time of 4.81 min for PAR, 2.80 min for CAF and 6.71 min for CAR by which giving good separation, well defined peak with more number of theoretical plates.

The flow rate was optimized with (0.8, 1.0, 1.5 and 2 mL min^{-1}). At 0.8 mL min^{-1}, there is no peak appeared in the chromatogram with 3 replications. This is attributed to the insufficient flow rate to elute PAR, CAF and CAR through the column. However, a significant difference was observed among all the rest flow rates. Based on the results obtained, 1 mL min^{-1} showed the best results in terms of peak area and retention time.

3.2. Analytical method validation

3.2.1. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and excipients. There was no interference due to the excipients at the retention time of PAR, CAF and CAR in blank and sample. Chromatograms of the blank and spiked sample are presented in Fig. 5-10.

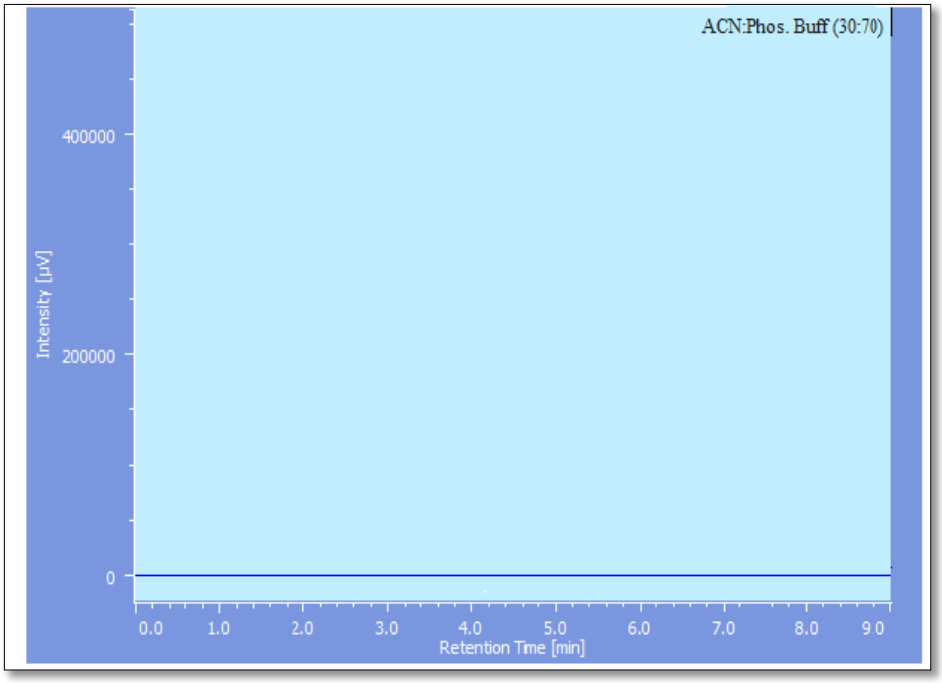

Fig. 5. Chromatogram for blank

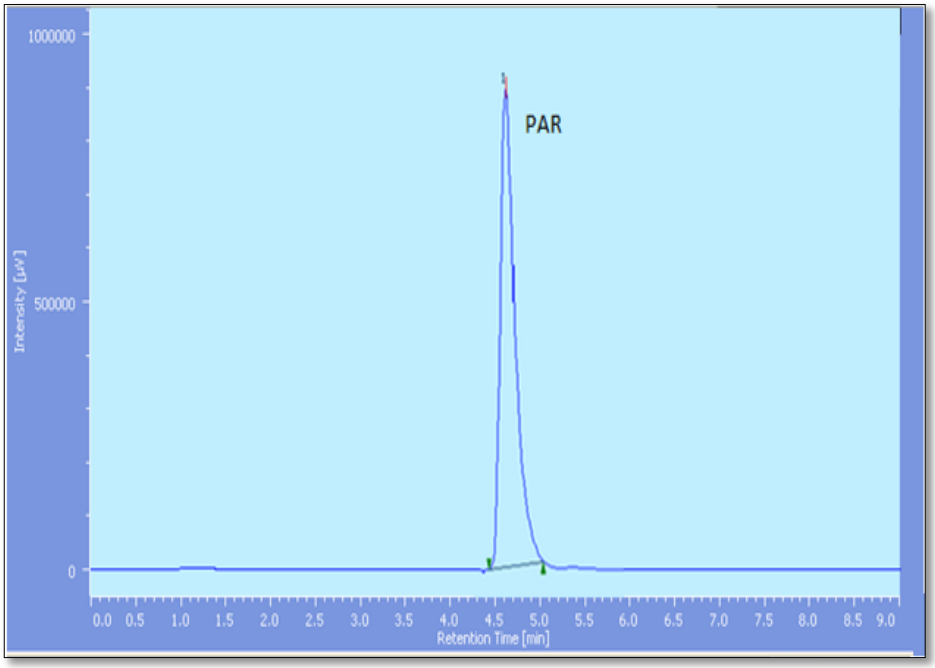

Fig. 6. Chromatogram of PAR at 5 µg mL^{-1}

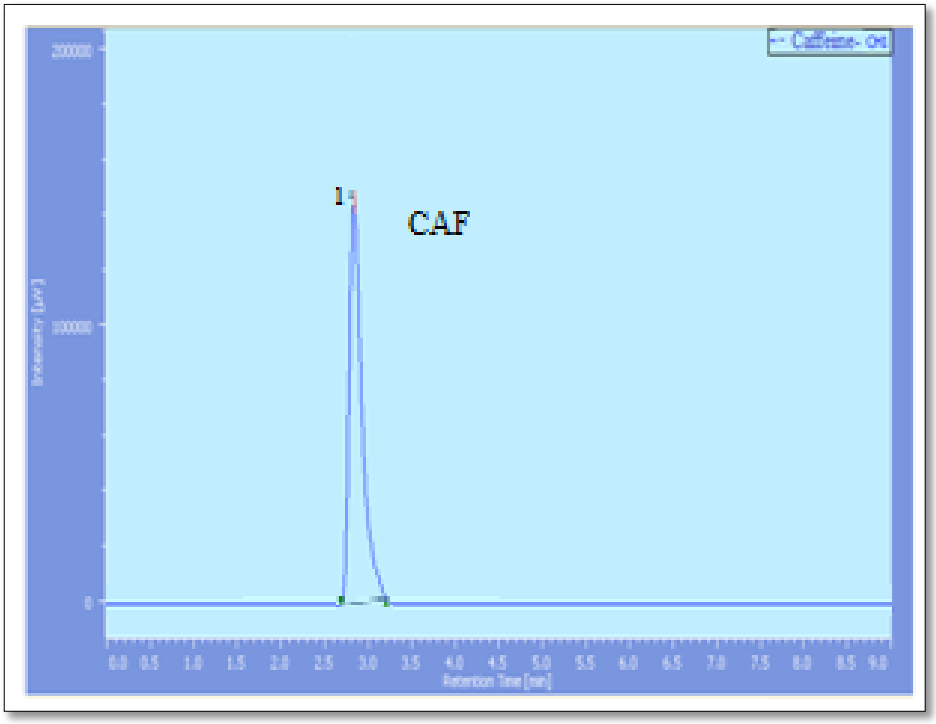

Fig. 7. Chromatogram of CAF at 5 µg mL^{-1}

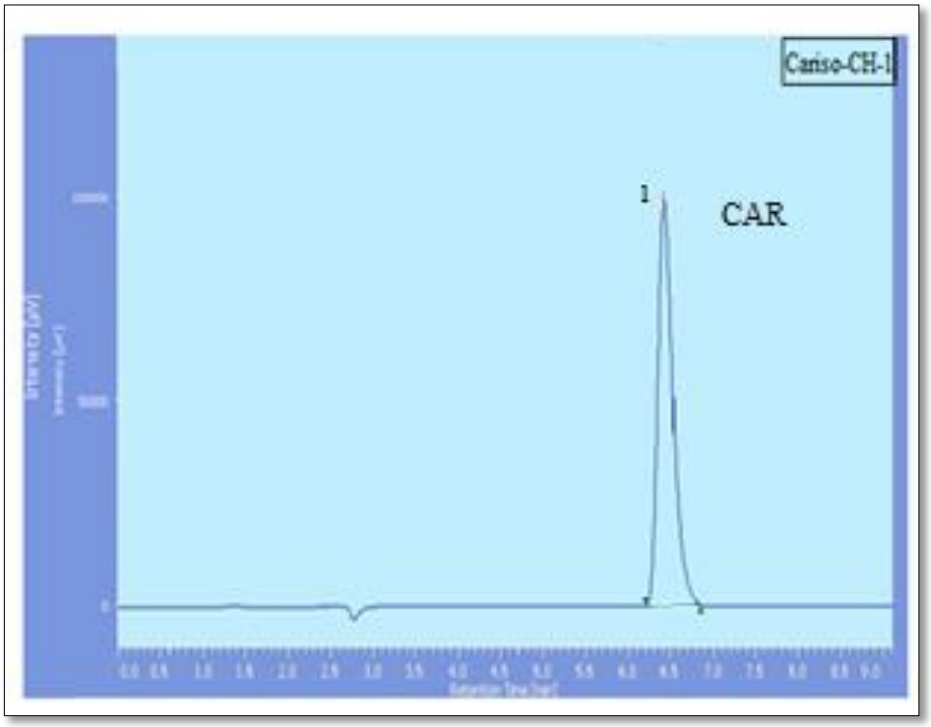

Fig. 8. Chromatogram of CAR at 5 µg mL^{-1}

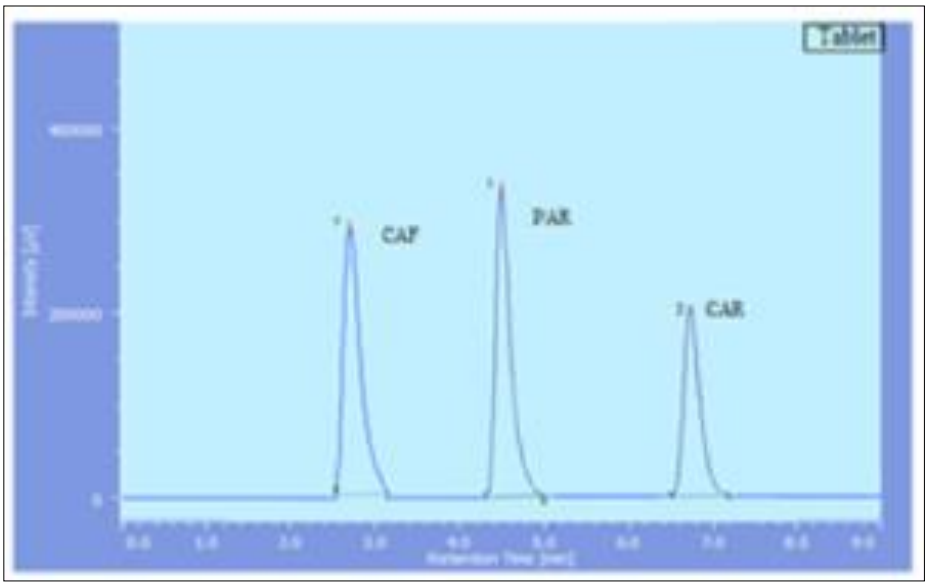

Fig. 9. Chromatogram of Tablet Solution

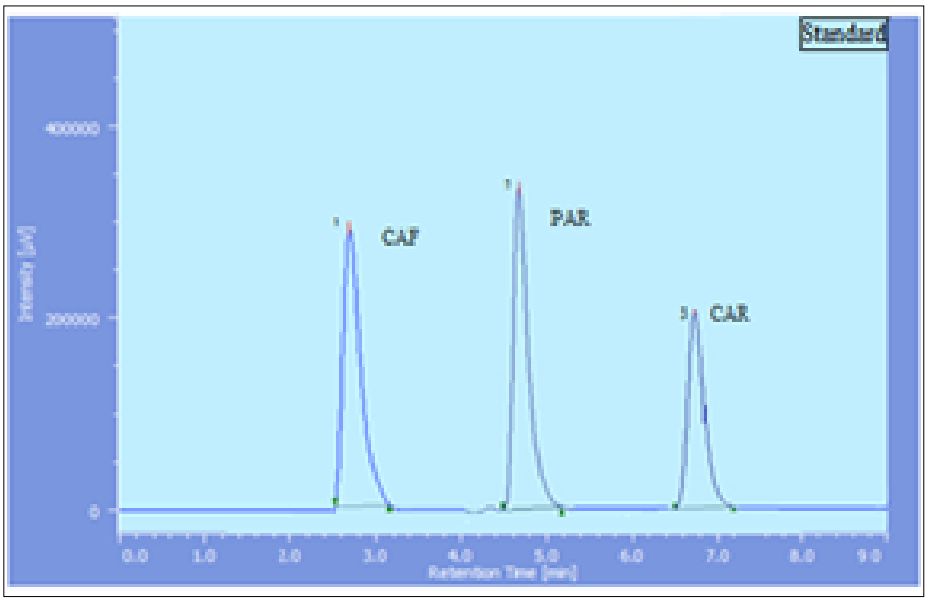

Fig. 10. Chromatogram of working standard of CAF**,** PAR and CAR

3.2.2 Linearity

Linearity study for the proposed method was established by least square linear regression analysis. The linearity of the method was determined by constructing calibration curves. Standard solution of the PAR, CAF and CAR of different concentration range (5-25 µg mL^{-1}, 5-25 µg mL^{-1}, 10-50 µg mL^{-1}respectively) were used for this purpose. Each measurement was carried out in five replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients which are presented in Table 3-5.

Table 3. Linearity data of PAR

Replicates	Standard conc. µg mL^{-1}					Regression Eqn.	R^2
	5	10	15	20	25		
	Peak area (µv/sec)						
1	587683	1206006	1842850	2402485	2851668	y=11780x	0.994
2	587726	1206011	1843332	2402425	2851993	y=11781x	0.994
3	587814	1206101	1843236	2402565	2852659	y=11783x	0.994
4	587840	1206233	1843065	2402542	2852092	y=11781x	0.994
5	587880	1206280	1842742	2402723	2852095	y=11781x	0.994
Mean	587788	1206126	1843045	2402548	2852101	y=11781x	0.994
±SD	81.71	125.91	249.54	111.85	357.29	-	-
RSD	0.0139	0.0104	0.0135	0.0046	0.0125	-	-

Table 4. Linearity data of CAF

Replicates	Standard conc. µg mL^{-1}					Regression Eqn.	R^2
	5	10	15	20	25		
	Peak area (µv/sec)						
1	104555	241644	351268	467984	558588	y= 22688x	0.995
2	104582	241643	351444	467856	558472	y = 22680x	0.995
3	104618	241634	351245	467865	558462	y = 22678x	0.995
4	104654	241748	351335	467832	558042	y = 22657x	0.995
5	104523	241755	351472	467742	555892	y = 22575x	0.994
Mean	104586	241784	351352	467855	558431	y = 22675x	0.995
±SD	51.46	61.06	102.04	86.65	222.12	-	-
RSD	0.0492	0.0252	0.0290	0.0185	0.0404	-	-

Table 5. Linearity data of CAR

Replicates	Standard conc. µg mL^{-1}					Regression Eqn.	R^2
	5	10	15	20	25		
	Peak area (µv/sec)						
1	1094213	2212455	3423939	4224432	5318921	y = 20922x	0.996
2	1093451	2213923	3424232	4223214	5319325	y = 20922x	0.996
3	1094382	2222132	3423215	4224239	5320923	y = 20910x	0.996
4	1093251	2214924	3424928	4225121	5313219	y = 20900x	0.996
5	1093962	2221131	3423121	4224623	5318935	y = 20906x	0.996
Mean	1093852	2216913	3423887	4224326	5318265	y = 20912x	0.996
±SD	486.54	4410.20	748.95	702.77	293.81	-	-
RSD	0.0444	0.1989	0.0218	0.0166	0.0166	-	-

3.2.3 Precision

Precision studies were carried out using analysis of drug by intra-day and interday variability. Results showed that the % RSD found less than 2. The precision study for PAR,

CAF and CAR was carried out with inter-day variability which is discussed in Table 6 and intra-day variability study was shown in Table 7.

Table 6. Inter-day variability of PAR, CAF and CAR

Conc. ($\mu g\ mL^{-1}$)	Peak area (µv/sec)			Mean area (µv/sec)	± SD*	RSD*
	Day 1	Day 2	Day 3			
PAR						
5	587683	587726	587814	587741	66.77	0.0113
15	1842850	1843332	1843236	1843139	255.12	0.0138
25	2851668	2851993	2852659	2852107	505.18	0.0177
CAF						
5	104555	104582	104618	104585	31.60	0.0302
15	351268	351444	351245	351319	108.86	0.0309
25	558588	558472	558462	558507	70.03	0.0125
CAR						
10	1094213	1093451	1094382	1094015	495.97	0.0453
30	3423939	3424232	3423215	3423795	523.50	0.0152
50	5318921	5319325	5320923	5319723	1058.68	0.0199

* indicates avreage of three determination

Table 7. Intra-day variability of PAR, CAF and CAR

Conc. ($\mu g\ mL^{-1}$)	Peak area (µv/ sec)			Mean area (µv/sec)	± SD*	RSD*
	Trial 1	Trial 2	Trial 3			
PAR						
5	587840	587880	587683	587801	104.12	0.0177
15	1843065	1842742	1842850	1842886	164.42	0.0089
25	2852092	2852095	2851668	2851952	245.66	0.0086
CAF						
5	104654	104523	104555	104577	68.29	0.0653
15	351335	351472	351268	351358	103.98	0.0295
25	558042	558592	558588	558407	316.39	0.0566
CAR						
10	1093251	1093962	1094213	1093638	507.62	0.0464
30	3424928	3423121	3423939	3423996	904.84	0.0264
50	5313219	5318935	5318921	5317025	3296.10	0.0619

* indicates avreage of three determination

3.2.4 Accuracy (Recovery study)

The accuracy (recovery study) was performed by the standard addition method. Three replicate injections, each of three different test concentrations in the range of 50, 100 and 150% were studied. The accuracy and reproducibility is apparent from the data as results are close to

100% and the value of standard deviation and % R.S.D were found to be < 2%, which shows the method is highly precise and accurate. The Recovery study of PAR, CAF and CAR was shown in Table 8.

Table 8. Recovery study of PAR, CAF and CAR

Recovery level %	Peak Area (µv/ sec)	Amt. Taken (µg mL^{-1})	Amt. added (µg mL^{-1})	Total amount (µg mL^{-1})	Amt. recovered (µg mL^{-1})	% recovery	Average recovery % ± SD	RSD
				PAR				
50%	983721	5.0	2.5	7.5	7.473	99.60	99.66 ±0.0650	0.065
	982857	5.0	2.5	7.5	7.482	99.73		
	983248	5.0	2.5	7.5	7.475	99.66		
100%	1206006	5.0	5.0	10	9.873	98.73	99.05 ±0.4067	0.410
	1206233	5.0	5.0	10	9.892	98.92		
	1206280	5.0	5.0	10	9.951	99.51		
150%	1424821	5.0	7.5	12.5	12.473	99.76	99.78 ±0.0493	0.049
	1423982	5.0	7.5	12.5	12.469	99.75		
	1423593	5.0	7.5	12.5	12.483	99.84		
				CAF				
50%	104523	5.0	2.5	7.5	7.512	100.13	99.90 ±0.2040	0.204
	104668	5.0	2.5	7.5	7.493	99.86		
	104582	5.0	2.5	7.5	7.480	99.73		
100%	241748	5.0	5.0	10	9.891	98.90	98.47 ±0.2563	0.262
	241634	5.0	5.0	10	9.803	98.03		
	241684	5.0	5.0	10	9.940	99.49		
150%	284932	5.0	7.5	12.5	12.551	100.13	99.71 ±0.0435	0.045
	284885	5.0	7.5	12.5	12.473	99.76		
	284974	5.0	7.5	12.5	12.420	99.33		
				CAR				
50%	1620452	5.0	2.5	7.5	7.469	99.46	99.56 ±0.1234	0.123
	1621984	5.0	2.5	7.5	7.478	99.70		
	1621876	5.0	2.5	7.5	7.465	99.53		
100%	2214924	5.0	5.0	10	9.893	98.93	98.92 ±0.3351	0.338
	2216853	5.0	5.0	10	9.925	99.25		
	2215728	5.0	5.0	10	9.858	98.58		
150%	2938242	5.0	7.5	12.5	12.422	99.36	99.51 ±0.0712	0.071
	2937873	5.0	7.5	12.5	12.435	99.44		
	2935985	5.0	7.5	12.5	12.439	99.51		

3.2.5 Limit of detection (LOD)

The limit of Limit of detection (LOD) for PAR, CAF and CAR was found to be 0.0051 µg mL^{-1}, 0.0150 µg mL^{-1} and 0.0406 µg mL^{-1} respectively.

3.2.6 Limit of Quantitation (LOQ)

The Limit of Quantitation (LOQ) for PAR, CAF and CAR was found to be 0.0157 µg mL^{-1}, 0.0456 µg mL^{-1} and 0.1232 µg mL^{-1} respectively.

3.2.7 Ruggedness

The ruggedness of the method was studied by special parameters like different laboratory condition, different analyst, different source of reagents and solution were used for the proposed method of estimation, as a result there was no significant change in the optimized parameters of the proposed method was observed. The ruggedness study has shown that there was no variation in the results of different laboratory condition, different analyst, different source of reagents and solution. The % RSD for ruggedness analysis was found to be less than 2. The Ruggedness data for PAR, CAF and CAR as indicated in Table 9.

Table 9. Ruggedness data for PAR, CAF and CAR

Parameter	% Assay			SD*			RSD*		
	PAR	CAF	CAR	PAR	CAF	CAR	PAR	CAF	CAR
Analyst - 1st	99.87	99.41	99.51	0.0351	0.5729	0.0862	0.0352	0.5763	0.0866
Analyst- 2nd	98.48	98.59	99.58	0.0602	0.0601	0.0529	0.0612	0.0611	0.0531
Lab-1st	97.93	98.40	99.84	0.0503	0.0801	0.0321	0.0513	0.0813	0.0321
Lab-2nd	99.30	96.33	99.53	0.0513	0.0458	0.0450	0.0516	0.0475	0.0453
Reagent - 1st	99.59	99.78	99.69	0.0305	0.0602	0.0404	0.0306	0.0604	0.0405
Reagent- 2nd	99.57	99.52	97.27	0.0305	0.0503	0.0450	0.0306	0.0505	0.0463

* indicates avreage of three determination

3.2.8 Robustness

The robustness of the analytical method is the measure of its capacity, to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The method must be robust enough to withstand slight changes and allow routine analysis of samples. Robustness of the method was determined by carrying out the analysis under conditions during which change in flow rate, change in the organic composition of the mobile phase, change in pH, and change in analytical wavelength was studied.

Variation of organic composition in the mobile phase, pH, wavelength and flow rate were seemed to have no significant impact on resolution, peak area, tailing factor, retention time and theoretical plates. The Robustness studies of system suitability parameter are discussed in Table 10-13.

Table 10. Robustness study of system suitability parameter: Change in flow rate (ml/min)

System suitability parameter*	Drug	Change in flow rate (ml/min)			RSD*		
		0.98	1.0	1.02	0.98	1.0	1.02
Peak area*	PAR	587657	587720	587818	0.0061	0.0013	0.0016
	CAF	104568	104636	104676	0.0182	0.0243	0.0148
	CAR	1094221	1094872	1093903	0.0010	0.0025	0.0058

Table 10. Continued

System suitability parameter*	Drug	Change in flow rate (ml/min)			RSD*		
		0.98	1.0	1.02	0.98	1.0	1.02
Theoretical plates*	PAR	2587	2521	2565	0.6013	0.3645	0.9372
	CAF	3135	3041	3250	0.5186	0.3487	0.3481
	CAR	3849	3878	3940	0.1653	0.2188	0.2871
Tailing factor*	PAR	1.537	1.482	1.530	0.4600	0.6679	0.3234
	CAF	1.430	1.423	1.407	0.7414	0.4969	0.3015
	CAR	1.320	1.330	1.318	0.8570	0.6908	0.5899
Retention Time*(Min)	PAR	4.865	4.820	4.838	0.4941	0.1467	0.1753
	CAF	2.818	2.808	2.818	0.3041	0.2014	0.2007
	CAR	6.882	6.728	6.818	0.2157	0.1156	0.1244

* indicates avreage of three determination

Table 11. Robustness study of system suitability parameter: Change in O.C. of M.P. Ratio

System suitability parameter*	Drug	Change in O.C. of M.P. Ratio			RSD*		
		75:25	70:30	65:35	75:25	70:30	65:35
Peak area*	PAR	587822	587758	557940	0.0168	0.0070	0.0121
	CAF	104828	104738	104817	0.0755	0.0735	0.0330
	CAR	1094437	1094785	1093514	0.0109	0.0060	0.0036
Theoretical plates*	PAR	2760	2870	2949	0.4099	0.3942	0.3356
	CAF	3253	3368	3460	0.2173	0.4408	0.3269
	CAR	3944	3920	3948	0.1613	0.2886	0.2149
Tailing factor*	PAR	1.560	1.537	1.650	0.1813	0.5058	0.1714
	CAF	1.483	1.460	1.463	0.5243	0.1937	0.3866
	CAR	1.330	1.333	1.321	0.2126	0.6365	0.7493
Retention Time* (Min)	PAR	4.828	4.838	4.848	0.1025	0.1169	0.1753
	CAF	2.818	2.837	2.810	0.2759	0.2741	0.4026
	CAR	6.840	6.831	6.837	0.1654	0.0828	0.9307

* indicates avreage of three determination, O.C.-Organic composition, M.P.-Mobile Phase

Table 12. Robustness study of system suitability parameter: Change in pH

System suitability parameter*	Drug	Change in PH			RSD*		
		3.0	3.1	2.9	3.0	3.1	2.9
Peak area*	PAR	597612	587887	578317	0.0070	0.0113	0.0182
	CAF	104833	104622	104634	0.0688	0.0946	0.0689
	CAR	1095369	1094266	1095368	0.0079	0.0067	0.0069
Theoretical plates*	PAR	2853	2832	2748	0.2974	0.4743	0.3344
	CAF	3247	3345	3415	0.0871	0.2747	0.1449
	CAR	3747	3875	3871	0.1887	0.1094	0.1096

Table 12. Continued

System suitability parameter*	Drug	Change in PH			RSD*		
		3.0	3.1	2.9	3.0	3.1	2.9
Tailing factor*	PAR	1.477	1.530	1.650	0.5264	0.5986	0.2772
	CAF	1.441	1.450	1.447	0.9320	0.2925	0.4396
	CAR	1.340	1.346	1.362	0.8967	0.5776	1.0383
Retention Time*(Min)	PAR	4.828	4.836	4.831	0.1757	0.1608	0.0878
	CAF	2.836	2.828	2.821	0.2742	0.2000	0.3509
	CAR	6.823	6.850	6.830	0.1036	0.1651	0.1552

* indicates avreage of three determination

Table 13. Robustness study of system suitability parameter: Change in Wavelength (nm)

System suitability parameter*	Drug	Wavelength (nm)			RSD*		
		230	232	234	230	232	234
Peak area*	PAR	597620	587612	587822	0.0052	0.0072	0.0169
	CAF	104825	104692	104942	0.0903	0.0810	0.0579
	CAR	1093364	1095678	1094588	0.0153	0.0327	0.0354
Theoretical plates*	PAR	2748	2759	2948	0.3344	0.1793	0.3117
	CAF	3431	3366	3557	0.2678	0.1050	0.1987
	CAR	3873	3759	3848	0.1825	0.0940	0.2388
Tailing factor*	PAR	1.542	1.508	1.540	0.5042	0.3751	0.2295
	CAF	1.470	1.471	1.447	0.1924	0.7696	0.4396
	CAR	1.353	1.358	1.338	0.1045	0.6248	0.6341
Retention Time* (Min)	PAR	4.829	4.842	4.821	0.1610	0.1606	0.2053
	CAF	2.808	2.822	2.828	0.3021	0.3006	0.1749
	CAR	6.840	6.869	6.871	0.1654	0.1132	0.1440

* indicates avreage of three determination

3.3 Solution stability study

Stability in solution was evaluated by the standard solution and the test preparation. The solution was stored at 5°C at ambient temperature without protection from light and tested after 12, 24, 36, and 48 hrs. The stability study of the stored standard solution and test preparation was performed and solutions were found to be stable for up to 48 hrs. The assay values obtained after 36 hr. were statistically identical with the initial value without measurable loss shown in Table 14.

Table 14. Solution stability of PAR, CAF and CAR

Drug	% Assay Initial	After 12 hrs.	After 24 hrs.	After 36 hrs.	After 48 hrs.
PAR	99.86%	99.80%	99.78%	99.74%	99.70%
CAF	100.5%	100.3%	100.1%	100.05%	100.0%
CAR	100.03%	99.98%	99.95%	99.92%	99.90%

4. Conclusion

The present work involved the development of accurate, precise, simple and suitable RP-HPLC method for estimation of the drugs in multicomponent tablet formulation. So in this dissertation a new RP-HPLC method described for simultaneous estimation of PAR, CAF and CAR. In the proposed RP-HPLC method, the estimation of PAR, CAF and CAR carried out by Acetonitrile: Buffer (0.1 mol L^{-1} Orthophosphoric acid) (30:70 v/v) as mobile phase, pH 3.1 at a flow rate of 1.0 mL min^{-1} and Hiq Sil C18 HS (4.6 x 250 mm) column. The detection of PAR, CAF and CAR was carried out at 232 nm. The retention time of PAR, CAF and CAR were found at 4.815 min, 2.804 min and 6.718 min respectively. The results of the analysis in the method were validated by ICH guidelines in terms of linearity and range, accuracy, precision, LOD, LOQ, ruggedness, robustness and solution stability from the studies it is concluded that the developed RP-HPLC method can be successfully used for the estimation of PAR, CAF and CAR in their combined tablet formulations. The developed RP-HPLC method is accurate, precise, sensitive, reliable, specific, reproducible, rapid and economical. No interference of additives or matrix is encountered in the developed method.

Acknowledgement

The authors are thankful to Principal, M. E. S. College of Pharmacy and secretary, Honorable Prashant Patil Gadakh, Mula Education Society, Sonai, for encouragement and availing of the necessary facilities during the course of investigation. Authors are also gratified to Watson Pharmaceuticals Pvt. Ltd., Goa, India for providing a gift sample of Carisoprodol.

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