Development and Validation of Novel GC-FID Method for Simultaneous Quantification of Paracetamol and Caffeine in Formulations

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Summary: The advancements in analytical techniques have enabled the development of highly sensitive and accurate methods for the identification of materials up to trace levels using microliter of sample. A highly sensitive and novel GC-FID method has been developed and reported herein for the simultaneous quantification of paracetamol (PCM) and caffeine (CAF) in samples up to trace levels. The method was validated as per ICH guidelines for its sensitivity, linearity, robustness, inter and intra-day variations, limit of quantification (LOQ), limit of detection (LOD), matrix effect, carryover, precision, and accuracy. The LOD of PCM and CAF were determined to be 100 ppm and 10 ppm whereas, LOQ of PCM and CAF were found to be 300 and 30 ppm respectively. The method was applied to quantify PCM and CAF in marketed tablets and dissolution samples of the tablets, which showed highly precise and accurate quantification of PCM and CAF. Moreover, the GC-FID method was compared with reported HPLC method which suggested the superiority of GC-FID method in performance and sensitivity. In short, the newly developed GC-FID method showed sensitivity and selectivity for PCM and CAF estimation and can be adopted as an efficient method for various applications.

Key Words: Paracetamol; Caffeine; GC-FID; Simultaneous method.

Introduction

Paracetamol (PCM) is an over the counter, a non-narcotic analgesic that is therapeutically used for minor to intermediate level of pain. Chemically, PCM is N-(4-hydroxyphenyl) acetamide with molecular weight 151.163 g/mol, as shown in **Error! Reference source not found.** (A), and is a pharmacologically active metabolic product of phenacetin and is the major constituent that produces painkilling effects. A minor but highly active metabolite (*N*-acetyl-*p*-benzoquinone) is very dangerous and is responsible for major adverse effects of the drug because it is very toxic to both liver and kidney. The half-life of PCM is 2–3 h and is relatively unaffected by renal excretion. With toxic doses or liver disease, the half-life may be increased two-folds or even more [1].

Caffeine (CAF) is widely used in combination with analgesics as it enhances their efficacy *via* an unknown mode of action. Most probably it competes at some receptors that produce different pharmacokinetic behavior to the PCM that leads to its increased pain killer efficacy [2]. CAF contains **purine** moiety which is a naturally occurring xanthine derivative [3]. Chemically CAF is 1,3,7-Trimethyl-1H-purine-2,6(3H,7H)-dihydro-1,3,7-trimethyl-1H-purine-2, 6-dione with molecular weight 194.19 g/mol and its structure is given **Error! Reference source not found.** (B). CAF is available in adjunct with other drugs for the treatment of many problems like; in combination with ergotamine (Cafergot) and phenobarbital for many headache issues. It is also used in combination with ephedrine to reduce weight and CNS stimulation [4].

Several analytical methods have been reported for quantification of PCM and CAF simultaneously in pharmaceutical and plasma samples using voltammetric analysis [5, 6], flow injection analysis [7], UV photometric analysis [8], spectrofluorometric analysis [9], LC-ESI-MS analysis [10], HPLC analysis [11] and RP-HPTLC analysis [12]. Whereas, most of the HPLC and RP-HPLC methods reported are for detection of PCM and CAF along with other drugs like aspirin [13, 14], ascorbic acid [15] ibuprofen [16], chlorpheniramine [17, 18] and guaifenesin [19]. However, all these methods present some limitations like, relatively large injection volume, preparation of complex solvent system as mobile phase e.g. in HPLC and process time, which needs to be addressed through a sophisticated method showing more reliability in simultaneous detection of PCM and CAF only in formulations.

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Gas chromatography (GC) is amongst the efficient analytical technique used for all forms of matter soluble in the organic solvents and has a wide range of molecular weights. The superiority of GC is due to 1) high level of separation due to high-efficiency capillary column; 2) accuracy for the quantitative analysis is very high with least deviation; 3) small sample amount used for analysis and 4) cost-effective in terms of low time consumption. The separation of the compounds through GC is very good, as efficient capillary columns are used for this purpose [20]. GC can be coupled with various detectors like mass spectrometry (GC-MS), flame ionization detector (GC-FID), etc., for the detection and quantification of compounds. FID, a detector coupled with the GC, can quantify the substance to trace level. The injection system with automation is a very good addition in this machine, it increases the accuracy of the results and decreases the errors related to manual sampling [21].

However, here we report a highly sensitive, efficient and stable GC-FID method for the simultaneous detection and quantification of PCM and CAF in formulation samples. The newly developed method was validated extensively under various conditions using ICH guidelines and successfully applied to available pharmaceutical formulations. The method reported here may find utility in both the pharmaceutical industry and forensic sciences for quantification to trace levels in the formulation and biological samples.

Experimental

Paracetamol and Caffeine were provided by Shaheen Pharmaceutical (Pvt) Ltd. Swat, Pakistan. Methanol and HCl of analytical Reagent Grade by Fisher Chemical, USA were used. Commercial tablets were purchased from the local market.

Instrumentation and operating conditions

Gas chromatographic system 7890 B (Agilent Technologies US) equipped with an autosampler was used for method development and validation. The detection was done using flame ionization detector (FID) using hydrogen for flaming. The output signals were evaluated through GC Chem Station software version (Rev.B.04.03). The column used in GC was (DB-5MS) having specification 30 m length x 0.250 μm ID x 0.25 μm film thickness was procured from Agilent Technologies, USA. The column oven was programmed as follows: initial column oven temperature was 150°C, 1st temperature ramping was 20°C/min up to 180°C, 2nd was 5°C/min up to 230°C and last was 50°C/min to 300°C. The injector and detector temperature were kept at 240°C and 250°C respectively. The sample was injected through autosampler at volume 1.μL and methanol was used as a cleaning agent between samples. Nitrogen served as carrier gas at a flow rate of 30.4 mL/min. The ratio was set at 50:1. Hydrogen gas and air were used for the flame of FID at a flow rate of 40 mL/min and 400 mL/min respectively. To check the system suitability and optimizing the operating conditions the injection volume was changed from 1 μL to 4 μL and injector temperature was changed from 240˚C to 260˚C.

Preparation of standard and sample solutions

Different solvents including water, hexane, methanol, and chloroform were used to check the solubility of PCM and CAF. Out of these, methanol was found to be the best solvent for both PCM and CAF. Stock solutions of PCM and CAF having a concentration of 2000 ppm were prepared by dissolving 20 mg of each separately in 10 mL of methanol. Calibrators containing the mixture of PCM and CAF were made from their respective stock solutions relative to the concentration ratios that are available in the tablet formulation (500 mg PCM and 50 mg CAF). Eight calibrators were made having a concentration range of PCM and CAF 10:1 ppm to 1500:150 ppm respectively. Two positive quality control concentrations levels were selected for both PCM and CAF, one containing lower-level concentration while others containing higher-level concentration that falls within the range of the calibration curve, were made separately from the standard not from the stock solutions mentioned above, to check the method accuracy and detection capacity. One negative quality control sample was also included that contains only the solvent but no PCM or CAF. It was only for the confirmation purpose in the method validation.

The test solution was prepared by grinding the formulated tablets (500 mg PCM and 50 mg CAF) in mortar and pestle. The powdered tablet was dissolved in methanol using the ultrasonic bath for 20 min till clear solution was prepared. The solution was filtered using a syringe filter (0.22 μm) prior to injecting it into GC-FID.

Analytical procedure

1.0 μL of blank methanol, six replicate injections of system suitability solutions and test sample solutions were separately chromatographed under the similar operating conditions.as described above. A resolution of not less than 3 between any two peaks was set as system suitability criteria. The RSD% of not more than 5% was set as system precision and accuracy criteria.

Method development and validation

The validation of the newly developed method was proceeded as per International Conference on Harmonization (ICH) Q2R1 guidelines for the parameters given below:

Calibration Model

Calibrators of the working range were run on the GC-FID to check their linear response compared to the concentration. Six calibrators of PCM and CAF having a concentration of 100:10, 300:30, 500:50, 700:70, 1000:100 and 1500:150 ppm respectively, were used and their relevant responses were plotting to obtain calibration line.

Precision and accuracy

Two types of precisions were checked including within run and between run having a wide concentration range. The set of calibrators in the working range was run three times on the same day, later the same set was repeated for three days. Two quality control positive samples with known concentrations for each set of runs were used to check the accuracy of the system. Moreover, inter and intra-day analysis was performed by injecting 6 replicates of samples to verify the precision/accuracy of the developed method.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The minimum amount of the PCM and CAF to be detected by the method was determined using the diluted sample and was confirmed by running it in the triplicate form. Limit of detection (LOD) and limit of quantification (LOQ) was calculated using formulas LOD = 3.3 σ/S and OQ = 10 σ/S respectively. Here, σ represents the standard deviation of response and S is the slope of the calibration curve. The acceptable limit for LOQ was the response of the required peak that should be 10 folds higher than the baseline or it should be three times more than LOD [22].

Robustness

A uni-variant method was applied to check the robustness of the method. The volume of injection was selected as one of the criteria to check its effect on the analytical method. Volume was reduced to half and increased to double than the usual volume of injection i.e. 1 µL and 4 µL respectively. The second criterion was the temperature of the inlet, which was set at 240ºC instead of 250ºC.

Carryover

The amount of PCM and CAF was increased to a higher level than the working range of the calibrators used in the linearity. The blank methanol sample was checked after each highly concentrated sample of PCM and CAF for any carryover. The highest concentration ratio of both of these active ingredients that are free from the carryover effect in the methanol as determined for this parameter. The concentration was confirmed by running thrice on the same method [23].

Matrix Effect

Interference of inactive constituents which are mostly used in tablet manufacturing was determined by using tablet samples. Tablet was powdered and a small amount was extracted with methanol and analyzed on GC-FID following the same analytical conditions [23].

Method Application

The developed and validated method was applied to assay and dissolution studies of commercial tablets. The dissolution test for the tablet was performed using a paddle stirring assembly of the USP XXII apparatus II. The vessels were filled with 900 mL of 0.1N HCl and maintained the temperature of water at 37±1ºC. One tablet was placed in each vessel of the apparatus. Paddle speed was adjusted at 25 rpm. The sample (10mL) was drawn out at a frequency of 30 min, 1, 2, 3, 4, 5, and 6 h intervals and was replaced with fresh solution. Each sample was extracted with methanol and dried. The samples were reconstituted in 1mL of methanol and run on the GC-FID for analysis on the method developed and described below [24]. The samples were also analyzed through HPLC to compare the efficiency of the newly developed GC-FID method.

Statistical Analysis

All the experiments were performed as triplicate and results are presented as mean ± standard deviation.

Results and Discussion

Optimization of chromatographic conditions

During the method development phase, several chromatographic parameters were optimized to obtain an acceptable peak shape and resolution between the two peaks with acceptable recoveries to satisfy the system suitability. The GC-FID method was developed by using Agilent column (DB-5MS) having specification -60°C to 325°C/ (350°C) 30 m x 0.250 µm x 0.25 µm in which nitrogen was used as carrier gas at a continuous flow rate of 1 mL/min. Inlet was used at split mode with a split ratio of 50:1. Hydrogen gas was used for the flame of the FID at a flow of 50 mL/min in the presence of air at a flow rate of 400 mL/min. Nitrogen gas at 30.4 mL/min was used as make-up gas for the flame of the FID. The temperature programming of the column oven was changed gradually to properly separate peaks of PCM and CAF. The best temperature programming which produced efficient elution and separation was achieved when the column was started from 150ºC then it was programmed 20°C/min to 180°C for 0 min with total run time was 13.9 min.

System suitability criteria

The system suitability criteria were defined on the basis of results obtained from several representative chromatograms at different operating conditions of injector volume and temperature as shown in Fig**.** 1. The suitable volume of injection was found to be 1 µL and injector temperature was 240˚C. The column efficiency determined from the analyte peak < 100000, resolution between two analyte peaks >10.0 and RSD% < 5. All the suitability parameters during validation and analysis of formulations were within the acceptable limits suggesting the suitability conditions.

GC-FID method validation

GC-FID method for the simultaneous quantification of paracetamol and caffeine was successfully developed based upon suitability criteria and validated using ICH guidelines on parameters stated below.

Linearity

The linearity of the system was established by plotting the graph between concentration and peak area of PCM and CAF standards and finding the correlation coefficient. The linearity was evaluated by running the 5 working standards of different concentrations. The range of linearity for PCM was 100-550 ppm and for CAF was 7-35 ppm as shown in Fig. 2.

Precision and Accuracy

A set of calibrators in the working range **was** used to draw a calibration model of PCM and CAF. The correlation coefficient was measured for each set of runs. Quality control levels were added to check the results using correlation of the concentrations and responses of the calibrators. To establish precision the calibrators set was run in the triplicate form to check inter-day and intra-day variation.

Inter-day Precision and Accuracy

A set of calibrators consisting of five levels, in the working range were run in the triplicate form in three consecutive days and the results proved that the method producing results with high precision and accuracy as shown below in the %RSD values in Table-1 and Table-2, these values were taken from the chromatograms and the calibration lines drawn after each set of run as shown in the Fig. 3A**.**

Intra-day Precision and Accuracy

A set of calibrators which consist of five levels, in the working range were run in the triplicate form on the same day and the results proved that the method producing results with high precision and accuracy as shown below in the %RSD values in Table-1 to Table-2 respectively, these values were taken from the chromatograms and the calibration lines drawn after each set of run as shown in the Fig 3B.

Recovery

A known amount of sample containing a mixture of PCM and CAF at a concentration ratio of 750:75 ppm was used to check the accuracy of the method. The percentage recovery of the PCM and CAF was determined and confirmed in the triplicate run which showed that the method has produced accurate results as shown in

Table-3 and their respective chromatograms in Fig **S2.**

LOD and LOQ determination

The LOD and LOQ for PCM and CAF were determined through the calibration curve method as per ICH Q2R1 guidelines. A series of diluted samples of known concentrations were injected to find standard deviation and slope of the regression line. The LOD of PCM and CAF were determined to be 100 ppm and 10 ppm and LOQ of PCM and CAF were found to be 300 and 30 ppm respectively as shown in Fig. 4.

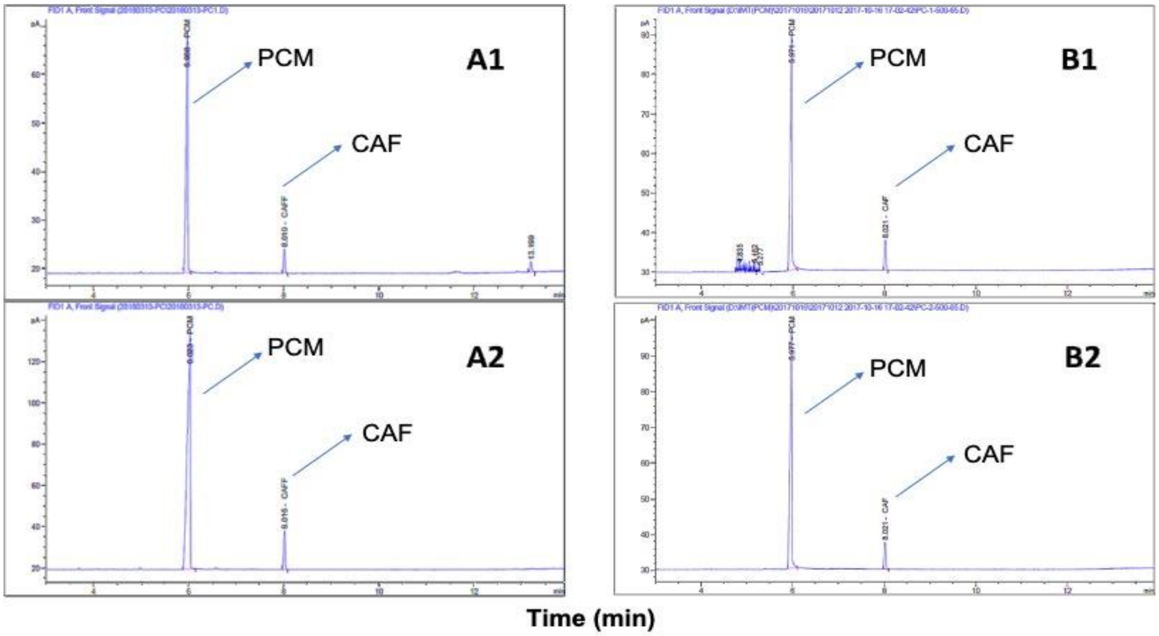

Fig. 1: Chromatograms of system suitability analysis showing peaks of Paracetamol and Caffeine at retention time 6 and 8 minutes respectively; (A1) injection volume 1µL; (A2) injection volume 4 µL; (B1) injector volume 240℃ and (B2) injector volume 260℃.

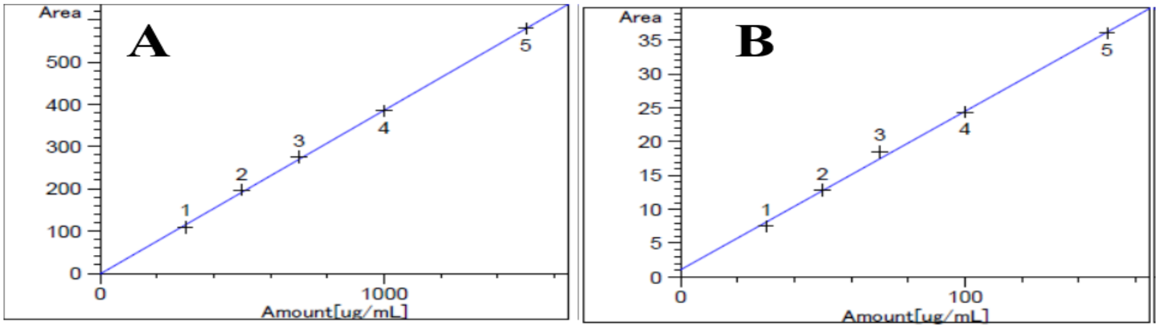

Fig. 2: Calibration curves of paracetamol and Caffeine labeled as A and B having correlation coefficient 0.99706 and 0.99737 respectively obtained from injection volume 1 µL introduced at 240℃ to the GC-FID.

Table-1: Triplicate runs on GC-FID of five calibrators of Paracetamol and Caffeine in the working range were run on the same day for inter-day precision and three consecutive days for intraday precision and data was used to calculate SD and %SD that show the correlation between concentration and response.

		Inter-Day					Intra-Day				
Sample/RT	**Amount (µg/mL)**	**R-1**	**R-2**	**R-3**	**Area (avg)**	**Amount/ Area**	**R-1**	**R-2**	**R-3**	**Area (avg)**	**Amount/ Area**
Paracetamol/5.96	300	109.257	102.86	93.637	101.917	2.944	102.859	101.699	101.811	102.123	2.937
	500	197.233	193.54	176.795	189.190	2.642	193.541	187.443	194.427	191.804	2.606
	700	275.365	273.09	260.939	269.798	2.594	273.089	275.357	274.994	274.479	2.550
	1000	383.893	380.83	367.077	377.266	2.650	380.827	387.674	385.295	384.598	2.600
	1500	579.726	586.04	586.229	583.997	2.568	586.035	592.026	586.230	588.097	2.551
					SD	0.151				SD	0.163
					%RSD	5.641				%RSD	6.171
Caffeine/8.019	30	7.523	7.407	6.799	7.243	4.142	7.407	7.443	7.436	7.429	4.038
	50	12.825	13.125	12.533	12.828	3.897	13.125	12.842	13.396	13.121	3.811
	70	18.514	19.274	18.877	18.888	3.705	19.274	19.600	19.596	19.490	3.591
	100	24.385	25.608	25.395	25.129	3.979	25.607	25.897	25.982	25.829	3.871
	150	36.011	37.917	38.066	37.331	4.018	37.917	38.321	38.066	38.101	3.936
					SD	0.161				SD	0.167

%RSD	4.093	%RSD	4.342

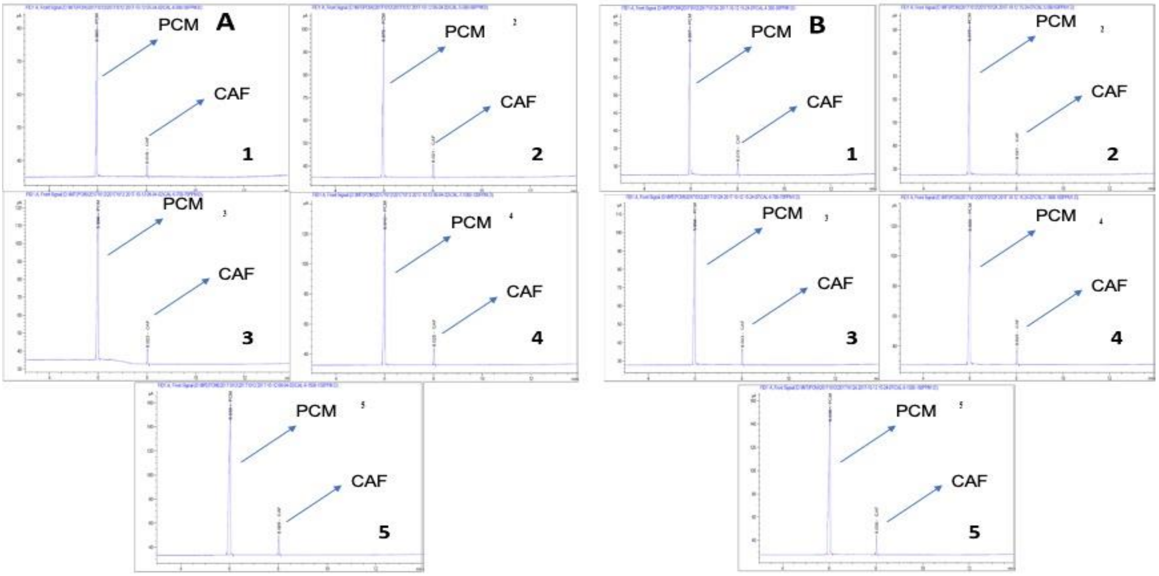

Fig. 3: Chromatograms of the mixture of Paracetamol:Caffeine calibrators 1-5 having concentrations of 300:30, 500:50, 700:70, 1000:100, 1500:150 ppm respectively, at retention time 6 and 8 min respectively, showing response proportional to the concentrations for (A) inter-day and (B) intra-day accuracy.

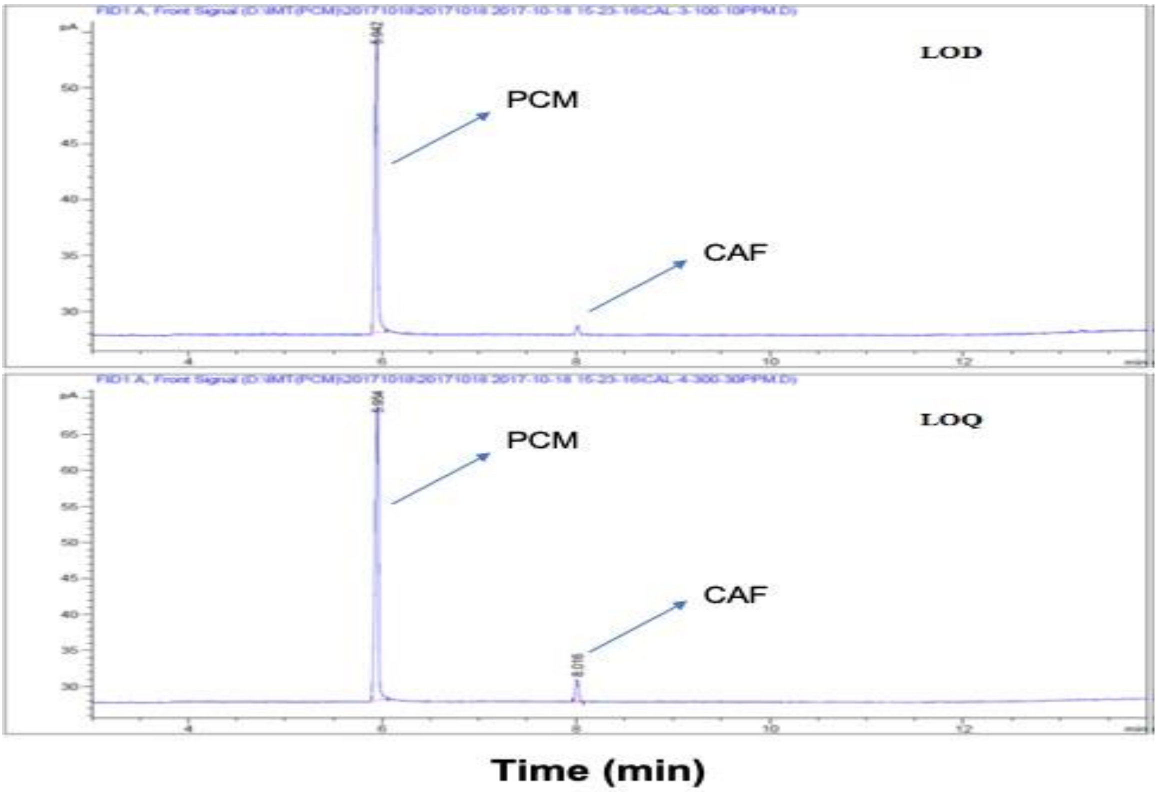

Fig. 4: (A) Limit of detection 100:10ppm and (B) Limit of quantification 300:30ppm for Paracetamol and Caffeine at retention at time 6 and 8 minutes respectively.

Table-2: Triplicate runs on GC-FID of two Quality Control positives of Paracetamol and Caffeine in the working range were run on the same day for inter-day accuracy and three consecutive days for intraday accuracy and data was used to calculate SD and %SD that show the correlation between concentration and response.

					Inter-Day					Intra-Day	
Sample/RT	**Amount (µg/mL)**	**R-1**	**R-2**	**R-3**	**Area (avg)**	**Amount/Area**	**R-1**	**R-2**	**R-3**	**Area (avg)**	**Amount/Area**
Paracetamol/5.96	**750**	**280.329**	**277.18**	**283.047**	**280.184**	**2.676**	**277.117**	**280.115**	**285.061**	**280.764**	**2.671**
	400	**144.955**	**132.57**	**137.601**	**138.374**	**2.890**	**132.569**	**128.560**	**141.171**	**134.100**	**2.982**
					SD	**0.151**				**SD**	**0.220**
					%RSD	**5.433**				**%RSD**	**7.792**
Caffeine/8.019	**75**	**18.594**	**19.137**	**19.365**	**19.032**	**3.940**	**19.137**	**19.120**	**19.440**	**19.232**	**3.899**
	52	**11.404**	**11.331**	**11.635**	**11.457**	**4.538**	**11.330**	**11.301**	**11.800**	**11.477**	**4.530**
					SD	**0.422**				**SD**	**0.446**
					%RSD	**9.973**				**%RSD**	**10.582**

Table-3: Triplicate run of known sample concentration ratio between Paracetamol and Caffeine 750:75 ppm respectively and their percentage recoveries.

Sr. #	**Amount of PCM/CAF (ppm)**	**Amount of PCM/CAF Recovered (ppm)**	**Percentage Recovery of PCM/CAF**
1	**750/75**	**724.10/73.90**	**96.54%/98.53%**
2	**750/75**	**737.64/74.56**	**98.35%/99.41%**
3	**750/75**	**741.05/74.21**	**98.80%/98.94%**

Carryover

Carryover concentration for the mixture of PCM and CAF was established by using the higher amounts that will overload the column and will appear in the next upcoming sample blank. Triplicate run of PCM and CAF having a concentration ration of 3000:300 ppm respectively along with their next sample blank contains traces of the drugs at retention time 6 and 8 as shown in Fig S3.

Matrix effect

The matrix effect on the method was checked and the excipients present in the tablet were run in the triplicate form that confirmed the stability and suitability of the method. It has produced acceptable results in the presence of other matrixes along with PCM and CAF at retention time 6 and 8 min respectively as shown in Fig S4.

Robustness

Method stability with minor changing of the internal parameters was determined and found that the method produced stable results even when the factors were disturbed. The uni-variate method was used for checking robustness, the volume of injection and inlet- temperature changes and results are shown in their respective chromatograms in Fig 1.

Application of the method

The developed and validated method was applied for the assay of PCM and CAF in the tablet sample. The results showed 100.54% of active ingredients in the tablets. This was further applied to the dissolution of tablet samples of paracetamol and caffeine for the simultaneous analysis of both the drugs and their relevant chromatograms were taken at a retention time of 6 and 8 min respectively as shown in Fig 5 and their amounts were calculated in **Error! Reference source not found.**. Furthermore, the sample was analyzed through HPLC (Fig S5) to see compare the sensitivity of both methods which showed superior sensitivity of the GC-FID method over HPLC.

With the advancement in analytical research, new and efficient methods for drug quantification up to ppm level are in high demand and are of scientific interest. This analysis is conducted through the latest equipment like LCMS, GCMS, and GC-FID, etc, resulting in efficient and accurate quantification of the drug in formulations as well as in plasma samples [25]. PCM and CAF combination is one of the most common and frequently consumed OTC medicines. The development of a highly sensitive method for the simultaneous quantification of PCM and CAF in samples would be really beneficial for the pharmaceutical industry and researchers. In this regard, a new GC-FID method was developed and validated for the simultaneous quantification of PCM and CAF in samples. The method was validated according to ICH guidelines.

The linearity studies were done on the method by analyzing three concentrations of each analyte on the developed method and then drawing the calibration line. The correlation coefficient (R^2) was calculated to be higher than 0.99. So, the method is perceived to be linear over the range of 100-500 ppm for PCM and 7-35 ppm for CAF. The peaks are ratio and the concentration of both drugs was subjected to regression analysis to calculate the calibration equations and correlation coefficient. The results showed an excellent correlation between the concentration and peak area ratio. The earlier discussed three mixed calibrators of PCM/CAF were prepared and selected for assay on GC-FID. The triplicate of each calibrator was run separately. A calibration curve was obtained from the data of the calibrators by using the offline soft wear of the GC-FID. Both the drugs showed a linear response relevant to the concentration of the solution.

All the mixed calibrators of PCM and CAF were analyzed in triplicates on the same day to measure any intra-day variability in the result on GC-FID. For inter-day variability, these calibrators were run on the GC-FID for three consecutive days. Peak areas were documented and the Relative Standard deviation (RSD) was measured statistically for both series of results obtained from the inter-day and intra-day calibrator runs separately**.** The % RSD value was <2 for all of the calibrators analyzed on the same day and on consecutive days. Calculations are compiled in the table form for inter-day precision as shown in while intra-day precision was compiled in Table-1**.**

The accuracy of the method was checked by using two known level concentration calibrators called low QC positive and high QC positive. One was of lower concentration and the other was of a higher concentration, but both fall in the range of the calibration curve so that the concentration can be accurately calculated from the calibration curve. Low QC positive having concentration of PCM and CAF 400:52 ppm respectively, while high QC positive having the concentration of PCM and CAF 750:75 ppm respectively. Triplicates of the QC positives were run along with other calibrators and their concentrations were calculated from their respective calibration curves. Percent (%) recovery of both components was calculated. The method found to be accurate for both of the components of the sample as values of QC positives were very good as shown in Table-2, and Fig 3.

Limit of detection (LOD) was measured by analyzing the different strengths of each drug and detection limit was established for both PCM and CAF. 100 ppm concentration was found to be LOD for PCM while 10 ppm was for CAF; below this concentration, both drugs did not provide response up to the acceptance criteria. The method proved to be quite sensitive for both PCM and CAF as it can analysis trace amounts of these drugs, as shown in Fig 4. From the experimental data LOQ calculated for PCM was 300:30 for PCM and CAF in this combination tablet dosage form.

Method validation may contain carryover as a parameter to be considered. For the determination of the carryover, a very concentrated sample of the analyte was injected, and it is followed by a blank sample to check is there any sample response available in the blank sample. The concentration at which carryover is available in the next blank sample is verified by repeating it three times. There should be no carryover in the blank sample when using method practically but if it is impossible to remove this problem then it should be documented and the response of the sample analyte should be ten times higher the carryover response present in the blank sample [23]. Carryover was observed at a concentration of 3000:300 ppm of PCM and CAF respectively which was checked by triplicate runs of the same as shown in Fig S3.

Inactive materials that are also part of the tablet formulation were analyzed along with active constituents of the tablet. Tablet excipients available in this tablet are PVP-K30, Magnesium Stearate, Talc, and HPMC. These expedients did not interfere with PCM and CAF during the analysis. The method found a stable in the presence of such impurities in the sample with no effects on the RT and response of the chromatogram.

For robustness study, the effect of minute, intended changes of parameters on the method was observed. The method was evaluated by changing the injection volume and the temperature ramping of the oven. After all these minute, intentional changes made with the method parameters, the method was found to be robust as shown in Fig **1.**

The method was applied to quantify the PCM and CAF in the commercial preparation and *in vitro* dissolution samples of the tablets. The method was sensitive enough to quantify the drug concentration in tablet formulation. The same samples were also analyzed through HPLC to compare the efficiency of techniques. The analysis of HPLC is very difficult to perform because of the hectic preparation phase before sample injection. No doubt samples with high concentration can be easily quantified through HPLC but trace level concentrated samples cannot be efficiently analyzed because of higher LOD of the instrument [26]. The response of PCM and CAF was very good on GC-FID with prominent and efficiently separated peaks even at a lower concentration which is not possible through HPLC. The similar solutions were analyzed using HPLC and the peaks produced by HPLC were not that sharp. Moreover, there were some ghost peaks appeared in the chromatograms. This suggested the better sensitivity of GC-FID in simultaneous detection of PCM and CAF from samples at trace levels. Detection of PCM and CAF in samples of dissolution studies at various time intervals can be compared on both techniques which showed that GC-FID is analyzing both of these drugs with better peak responses and sharpness, which can be checked comparatively in Fig S5. The amount of these two analytes at that specific time intervals proves that GC-FID gives the best response even at lower concentrations comparative to the HPLC that can be concluded by comparison.

Conclusion

A highly stable and sensitive simultaneous quantification method for PCM and CAF using GC-FID has been successfully developed and validated. The method showed high precision and accuracy for the qualitative and quantitative determination of PCM and CAF from samples up to the PPM level. The method is applicable not only for the evaluation of pharmaceuticals but also could be used for the trace level drug concentrations in blood. The method was evaluated by applying to the commercial formulations and was compared with HPLC, and the former showed better results in terms of LOD, peak sharpness and appearance of ghost peaks. LOD was much lower on GC-FID, peak sharpness, peak broadening, and ghost peaks were major issues in the chromatograms PCM/CAF as obtained by the analysis on HPLC. The method proves its efficiency and shows excellent potential to be applied in the pharmaceutical industry and research.

Conflict of Interest

The authors declare no conflict of interest.

References

1. M. M. D. H P Rang, J M Ritter, R J Flower, *Rang & Dale Pharmacology*. 6th ed. 2007: Churchill Livingstone Esevier 829.year
2. B. Renner, G. Clarke, T. Grattan, A. Beisel, C. Mueller, U. Werner, G. Kobal, and K. Brune, Caffeine accelerates absorption and enhances the analgesic effect of acetaminophen. *The Journal of Clinical Pharmacology*. **47**(6). (2007)
3. M. A. Heckman, J. Weil, D. Mejia, and E. Gonzalez, Caffeine (1, 3, 7 ‐ trimethylxanthine) in foods: a comprehensive review on consumption, functionality, safety, and regulatory matters. *Journal of food science*. **75**(3). (2010)
4. B. G. Katzung, *Basic and Clinical Pharmacology*. 2006, San Francisco: Mc Graw Hill Lage.year
5. B. C. Lourenção, R. A. Medeiros, R. C. Rocha-Filho, L. H. Mazo, and O. Fatibello-Filho, Simultaneous voltammetric determination of paracetamol and caffeine in pharmaceutical formulations using a boron-doped diamond electrode. *Talanta*. **78**(3). (2009)
6. M. Tefera, A. Geto, M. Tessema, and S. Admassie, Simultaneous determination of caffeine and paracetamol by square wave voltammetry at poly (4-amino-3-hydroxynaphthalene sulfonic acid)-modified glassy carbon electrode. *Food Chem*. **210**. (2016)
7. W. C. Silva, P. F. Pereira, M. C. Marra, D. T. Gimenes, R. R. Cunha, R. A. Da Silva, R. A. Munoz, and E. M. Richter, A simple strategy for simultaneous determination of paracetamol and caffeine using flow injection analysis with multiple pulse amperometric detection. *Electroanalysis*. **23**(12). (2011)
8. P. Ortega-Barrales, R. Padilla-Weigand, and A. Molina-Diaz, Simultaneous determination of paracetamol and caffeine by flow injection-solid phase spectrometry using C18 silica gel as a sensing support. *Analytical sciences*. **18**(11). (2002)
9. A. B. Moreira, I. L. Dias, G. O. Neto, E. A. Zagatto, and L. T. Kubota, Simultaneous spectrofluorimetric determination of paracetamol and caffeine in pharmaceutical preparations in solid ‐ phase using partial least squares multivariate calibration. *Anal Lett*. **39**(2). (2006)
10. A. Wang, J. Sun, H. Feng, S. Gao, and Z. He, Simultaneous determination of paracetamol and caffeine in human plasma by LC–ESI–MS. *Chromatographia*. **67**(3-4). (2008)
11. B. Tsvetkova, B. Kostova, I. Pencheva, A. Zlatkov, D. Rachev, and P. Peikov, Validated LC method for simultaneous analysis of paracetamol and caffeine in model tablet formulation. *International Journal of Pharmacy and Pharmaceutical Sciences*. **4**(4). (2012)
12. F. Soponar, A. C. Moţ, and C. Sârbu, Quantitative evaluation of paracetamol and caffeine from pharmaceutical preparations using image analysis and RP-TLC. *Chromatographia*. **69**(1-2). (2009)
13. B. J. Sanghavi and A. K. Srivastava, Simultaneous voltammetric determination of

acetaminophen, aspirin and caffeine using an in situ surfactant-modified multiwalled carbon nanotube paste electrode. *Electrochimica Acta*. **55**(28). (2010)

14. D. Šatínský, I. Neto, P. Solich, H. Sklenářová, M. Conceicao, B. Montenegro, and A. N. Araújo, Sequential injection chromatographic determination of paracetamol, caffeine, and acetylsalicylic acid in pharmaceutical tablets. *Journal of separation science*. **27**(7‐8). (2004)
15. O.-W. Lau, S.-F. Luk, and Y.-M. Cheung, Simultaneous determination of ascorbic acid, caffeine and paracetamol in drug formulations by differential-pulse voltammetry using a glassy carbon electrode. *Analyst*. **114**(9). (1989)
16. M. Khoshayand, H. Abdollahi, M. Shariatpanahi, A. Saadatfard, and A. Mohammadi, Simultaneous spectrophotometric determination of paracetamol, ibuprofen and caffeine in pharmaceuticals by chemometric methods. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. **70**(3). (2008)
17. M. Qi, P. Wang, Y. Leng, J. Gu, and R. Fu, Simple HPLC method for simultaneous determination of acetaminophen, caffeine and chlorpheniramine maleate in tablet formulations. *Chromatographia*. **56**(5-6). (2002)
18. A. Acheampong, W. O. Gyasi, G. Darko, J. Apau, and S. Addai-Arhin, Validated RP-HPLC method for simultaneous determination and quantification of chlorpheniramine maleate, paracetamol and caffeine in tablet formulation. *SpringerPlus*. **5**(1). (2016)
19. A. U. Kulikov and A. G. Verushkin, Simultaneous determination of paracetamol, caffeine, guaifenesin and preservatives in syrups by micellar LC. *Chromatographia*. **67**(5-6). (2008)
20. H. M. McNair and J. M. Miller, *Basic gas chromatography*. 2011: John Wiley & Sons.year
21. T. Nowak, G. C. Graffius, Y. Liu, N. Wu, X. Bu, X. Gong, C. J. Welch, and E. L. Regalado, GC-FID method for high-throughput analysis of residual solvents in pharmaceutical drugs and intermediates. *Green Chem*. **18**(13). (2016)
22. B. YÜKSEL and N. ŞEN, Development and validation of a GC-FID method for determination of cocaine in illicit drug samples. *JOURNAL OF RESEARCH IN PHARMACY*. **22**(4). (2018)
23. S. W. G. f. F. Toxicology, Standard practices for method validation in forensic toxicology. *J. Anal. Toxicol*. **37**. (2013)
24. *USP32-NF27*. Vol. 1. 2009, Baltimore, Maryland: United Book Press.year
25. I. Jerković, M. Kranjac, Z. Marijanović, M. Zekić, A. Radonić, and C. I. G. Tuberoso, Screening of Satureja subspicata Vis. honey by HPLC-DAD, GC-FID/MS and UV/VIS: prephenate derivatives as biomarkers. *Molecules*. **21**(3). (2016)
26. M. S. Carvalho, M. A. Mendonça, D. M. Pinho, I. S. Resck, and P. A. Suarez, Chromatographic analyses of fatty acid methyl esters by HPLC-UV and GC-FID. *J Braz Chem Soc*. **23**(4). (2012)