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Validation of a Method for Simultaneous Determination of Acetaminophen and Caffeine by HPLC in Different Pharmaceutical Forms: Tablet, Capsule and Sachet

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Abstract
A simple, fast, economical, accurate, precise and reproducible RP – HPLC method was developed for the simultaneous estimation of acetaminophen (AAPH) and caffeine (CAF) in starting materialand pharmaceutical dosage forms. The method was validated in terms of specificity, linearity, precision accuracy, and robustness. The proposed method's results were found to be satisfactory and are suitable for determination of acetaminophen and caffeine for routine quality control of drugs in formulations.

Keywords:acetaminophen, caffeine, HPLC, validation.

1. Introduction
Acetaminophen (AAPH), in figure 1, is the active ingredient of many drug specialties of the class of non-salicylate antipyretic analgesics. Chemically it is a N-(4-hydroxyphenyl)acetamide. It is indicated for the symptomatic treatment of fever and low to moderate pain, alone or in association with other analgesics. In contrast to non-steroidal anti-inflammatory drugs and in particular aspirin, it is devoid of anti-inflammatory properties and does not act on platelet aggregation. Caffeine (CAF), chemically described as 1,3,7-trimethyl-1H-purine-2,6(3H,7H)-dione (Fig. 2) is an alkaloid of the methylxanthine family, present in many foods, which acts as a stimulant psychotrope and as a mild diuretic.

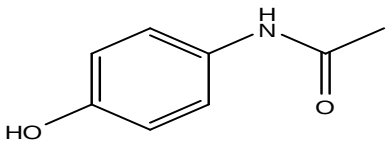


Figure 1: Structure of acetaminophen (AAPH)

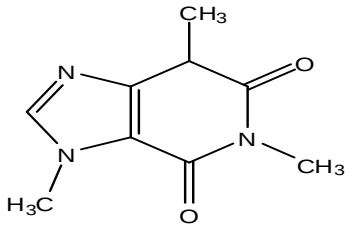


Figure 2: Structure of caffeine (CAF)

Acetaminophen and caffeine-based medicines are available in different pharmaceuticalforms; for example: Claradol 500mg Caffeine (Bayer Health Care), Exidol (Galephar), Theinol (Bailly-Creat). Currently, measurement of these molecules in the finished products is done through various methodssuch as UV, HPLC …

[1-17]. Hence, the present investigation was aimed at developing a fully validated HPLC-PDA(Photodiode Array) method for the simultaneous estimation of acetaminophen and caffeine in different pharmaceutical forms.
We propose a simple and rapid analytical method for the determination of these active ingredients in these pharmaceuticalforms[19,20]. The aim of such a move is to help laboratories and Drug specialist's pharmaceutical industry, to reduce the time and cost of analysis and subsequently minimize chemical releases.
In this work, we developed and validated according to ICH Q2B guidelines strategy [21] (International Conference on Harmonization), a simple and rapid RP-HPLC method. It is specific, linear, accurate, precise and robust.

2. **Experimental**

2.1. *Apparatus:*
The chromatographic systems used is constituted by a Waters 2695 pump, an auto sampler and a Waters 2998 PDA detector. Spectra Manager software and Empower Software data registration were used for all absorbancemeasurements.TheMettler Toledo scale was manufactured Switzerland.

2.2. *Reagents and standards:*
The standard used for the determination of acetaminophen is a working standard having a purity of 100.5% and the water content of 0.1% and that of caffeine was 99.8% purity and water content is 0.11%. The only reagent used is methanol which is HPLC grade was supplied from Sigma - Aldrich (Germany).
The placebo used in the validation process consists of the usual excipients present in the commercial formulation: povidone, colloidal anhydrous silica, magnesium stearate, sodium saccharinate, gelatine capsule, lactose and talc.

2.3. *Chromatographic conditions:*
The chromatographic conditions are gathered in the table 1.The mobile phase was filtered through a 0.45-µm Millipore filter and degassed by vacuum prior to use.

Table1: Chromatographic conditions of the method

Column	Symmetry RP18 Column, 150 mm × 4.6 mm, 3.5µm.
Flow rate	1 ml/min
Temperature	25°C
Wavelength	275nm
Injection volume	10 µl
Mobile phase	(Methanol / Distilled water): (30% / 70%)vol/vol
Dilution medium	Distilled water
Sample stability	24 hours at room temperature

3. Results and discussion

3.1 The specificity of the chromatographic method:
The specificity of the methodwasconfirmed by the absence of potentialinterferencecaused by the excipients withacetaminophen and caffeine, by comparing the chromatograms of the blank, placebo, active ingredientalone (AIA), and that of the reconstitutedpharmaceuticalform (FPR) (Figure 2a)
The purity angles of acetaminophen and caffeinepeaks are lowerthan the threshold (AAPH : 1.340 < 2.476; CAF: 0.281 < 0.285) (Figure 2b).This shows that the methodis capable of easilyassayedacetaminophen and caffeine in the presence of these excipients.

3.2 Linearity
The linearity of the method was determined by preparing 3 series of five minimum concentrations (70%, 85%, 100%, 115%, 130%) of the target concentration (500µg/mL of acetaminophen and 50µg/ml of caffeine) of the active ingredients alone (AIA) and reconstituted pharmaceutical form (FPR) (Fig. 3).

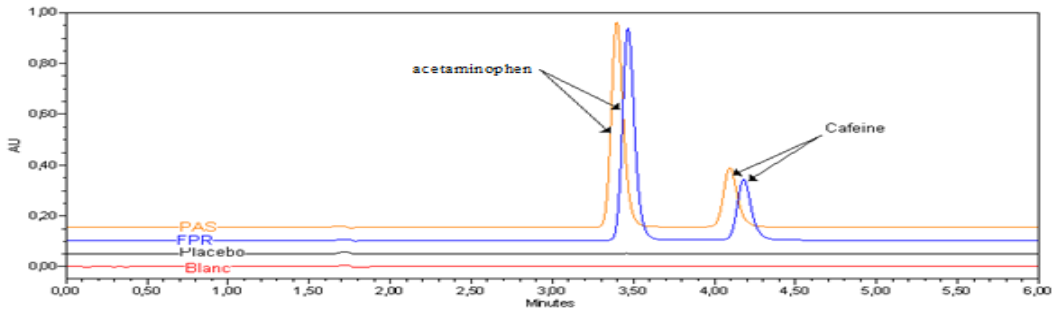


Figure 2a: Chromatograms of Blank, Placebo, AIA and FPR.

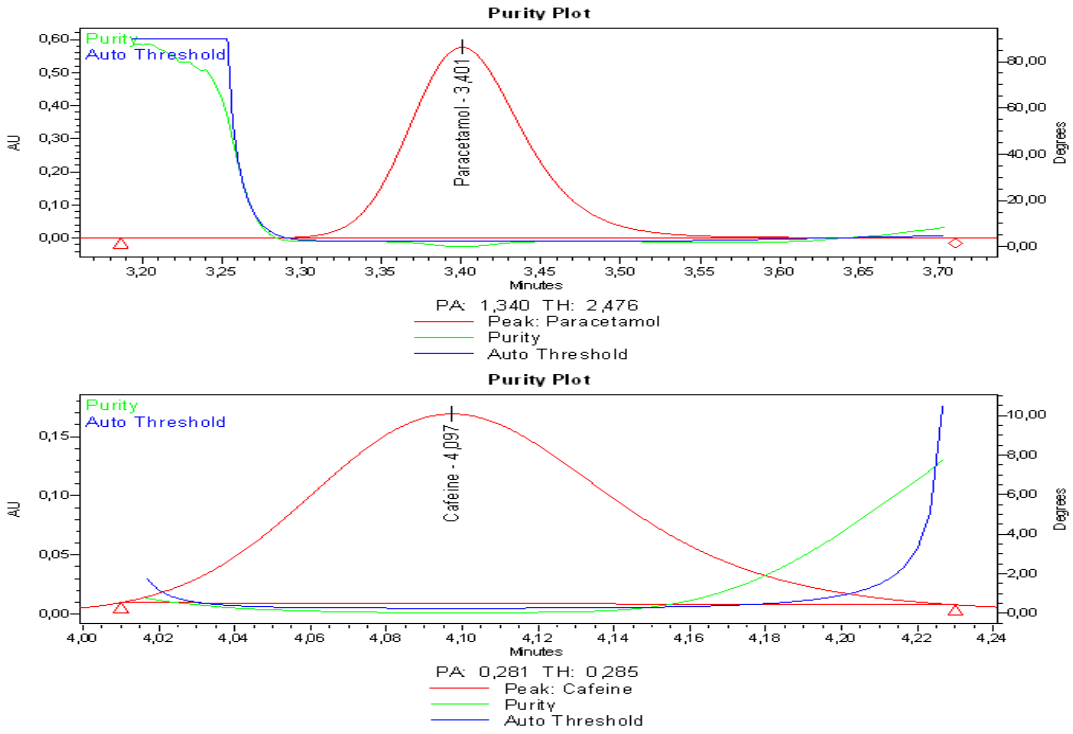


Figure 2b: Chromatographicpurities of AAPH and CAF

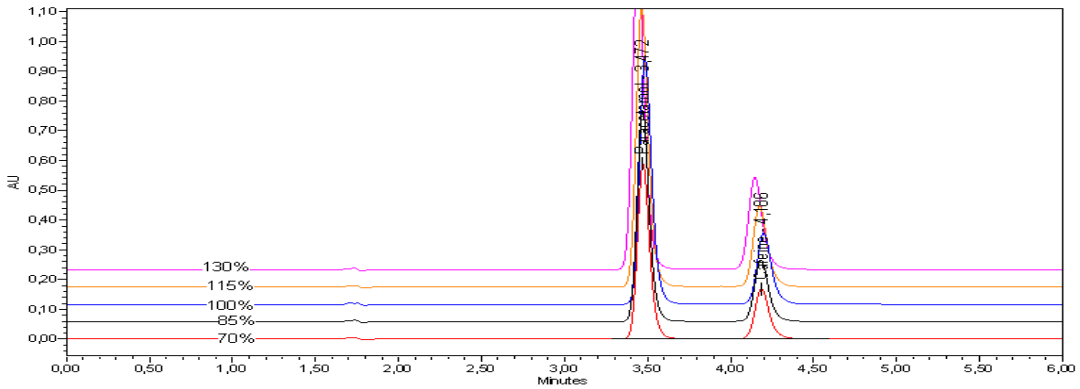
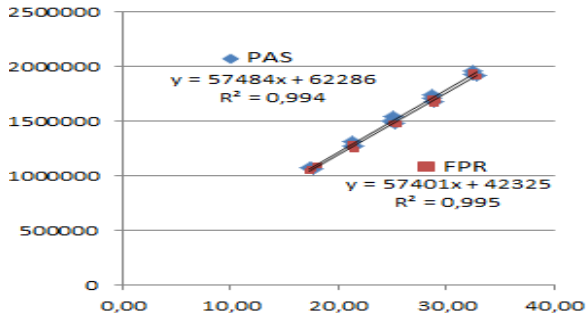


Figure3: Chromatograms of the linearity study of the method

The average of each injection zone and graphing the averagepeak relative to the actual concentration of each solution (Figures 4 and 5).The regression equations of AIAand FPR are linear for acetaminophen and caffeine (Table 2 and Figures 3, 4).



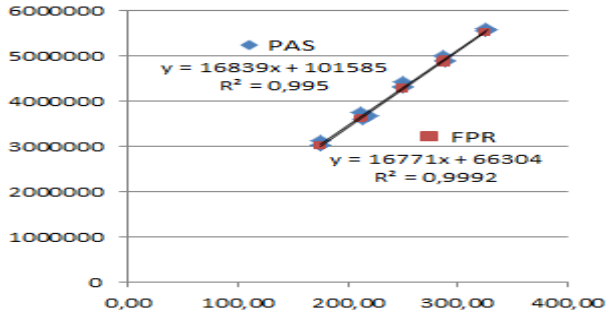


Figure 4: Linearity of CAF **Figure 5:** Linearity of AAPH

Table 2: Study of the linearity of the method.

	CAF		**AAPH**	
	PAS	FPR	PAS	FPR
Regression equations	y=57484x + 62286	y=57401x + 42325	y=16839x + 101585	y=16771x + 66304
Slope : **a**	57484	57401	16839	16771
Intercept: **b**	62286	42325	10158	66304
Correlation coefficient: $\mathbf{r^2}$	0.994	0.995	0.995	0.999
Confidence interval of **a**	Max=60069.47 Min=54938.28	Max=59738.00 Min= 55114.96	Max=17542,79 Min= 16138.53	Max=17039.59 Min=16502.92
Confidence interval of **b**	Max=127479.85 Min= -3883.28	Max=101105.75 Min= -17720.41	Max= 281074.85 Min= -78787.95	Max=134809.50 Min= -2451.48

3.3 Precision (repeatability and intermediate precision).
The repeatability and the intermediateprecisionwerevalidated as described in the ICH guidelines Q2B [16] by performing 3 series (3 differentoperators) of six sampleseachcontaining 100% of active ingredients in a reconstitutedpharmaceuticalform. (Figure 6).

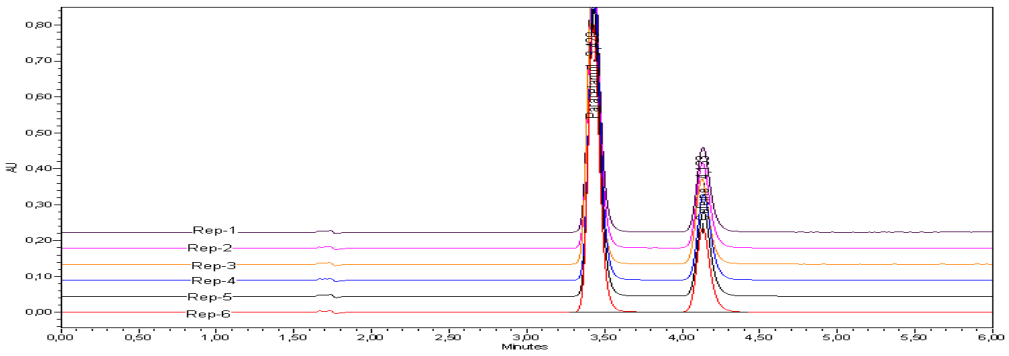


Figure 6: Chromatograms of the repeatability study of the method.

The relative standard deviations of repeatability and intermediateprecision of the two active ingredients are lessthan 2% (Table 3), and wecanconcludethat the analyticalmethodchosenisprecise.

Table3: Study of the precision of the method.

	CAF	**AAPH**
Repeatability	RSD_r = 0.95%	RSD_r = 0.57%
Intermediateprecision	RSD_{IP} = 0.89%	RSD_{IP} = 0.53%

3.4 Accuracy

The accuracy of the methodwasdetermined on 3 series (3 different operators) of their constituted pharmaceutical form having five concentration levels (70%, 85%, 100%, 115% and 130%) of the target concentration. The averagerecovery of the two active ingredients and the confidence interval of the measurement are reported in Table 4. They are all included in the standard values fixed by the EuropeanPharmacopoeia (95% and 105%), therefore the method of assay is accurate.

Table4: Study of the accuracy of the method.

	CAF	**AAPH**
Average recovery	99.18%	98.93%
Confidence interval	[98.19 - 100.17]	[98.52 – 99.34]

3.5. Robustness

The robustness of the method was studied by changing several experimental parameters: the changes in the method such as changing the eluant flow rate of 0.1 mL/min that is to say a value of 0.9 ml/min and 1.1 ml/min), the temperature of the column (5°C of the set point namely : 20°C and 30°C), the composition of the mobile phase (methanol/distilled water) (25/75% and 35/65%), the detection wavelength (272 and 278 nm) and different column trademarks (Table 5). From the results of the number of theoretical plates, asymmetry factor, resolution, content and RSD, due to the variation of these parameters, we can conclude that the method is robust.

Table 5: Robustness of the method.

	Number of TheoreticalPlates N > 3000		**Asymmetry Factor** 1.1%< F< 1.5%		**Resolution** > 3.5	**Content** (95% to 105%)		**RSD** < 2.5%	
	AAPH	**CAF**	**AAPH**	**CAF**		**AAPH**	**CAF**	**AAPH**	**CAF**
Temperature**20°C**	9580	11080	1.19	1.18	4.9	99.51	99.35	0.2	1.4
Temperature**25°C**	8980	10360	1.17	1.17	5.0	99.85	101.33		
Temperature**30°C**	11900	11920	1.18	1.15	4.9	99.92	98.73		
Mobile phase **75/25%**	10670	12070	1.15	1.14	7.5	99.17	97.73	0.4	2.2
Mobile phase **70/30%**	11900	10360	117	1.17	5.0	99.85	101.33		
Mobile phase **65/35%**	8930	9910	1.20	1.22	4.1	99.13	97.43		
Flow = **0.9 ml/min**	10890	11030	1.16	1.15	5.0	98.82	99.54	0.6	1.1
Flow = **1.0 ml/min**	11900	10360	1.17	1.17	5.0	99.85	101.33		
Flow = **1.1ml/min**	10620	11290	1.15	1.15	5.0	100.00	99.35		
λ= **278nm**	8990	10220	1.10	1.17	5.0	101.45	102.02	1.8	0.4
λ= **275nm**	11900	10360	1.17	1.17	5.0	99.85	101.33		
λ= **272nm**	8980	10240	1.10	1.18	5.0	103.53	102.07		
Waters Column	11900	10360	1.17	1.17	5.0	99.85	101.33	2.1	1.4
SunFireColumn	10850	15785	1.05	1.15	16.8	103.39	99.62		
KromasilColumn	9990	11600	1.32	1.36	14.1	99.54	98.49		

Though our study of validation of a new method is to determination of acetaminophen and caffeine by HPLC in different pharmaceutical forms.It is capable of simultaneously dosing acetaminophen and caffeine with a good resolution (> 3.5).

Besides the mobile phase prepared by 70% distilled water, the retention time is relatively short (less than 4.5min), which minimizes the amount of chemical discharges. It is an environment-friendly methodand it will save considerable analysis time.

Conclusion

The proposed RP-HPLC - PDA methodwasvalidatedfully as per International Conference on Harmonization (ICH) Guidelines, and found to be applicable for routine quality control analysis for the estimation of AAPH and CAFincombinationusingisocratic mode of elution.
The assaymethodproposed RP-HPLC wasdemonstrated as a simple, rapid and economical.
The mobile phase consisted of 70% of distilled water, simple to prepare and the analysis time islessthan 6 min consumes lessthan 6 ml of mobile phase, the flow rate is 1 ml/min, the preparation of the samplesiscarried out in water only.
The validation of the methodisbased on a statisticalstudy (Cochran's test, Student's test, Fisher test, Dixon'stest...). The methodisspecific, linear, accurate, faithful and robust. Therefore, thismethodcanbeemployed in quality control to estimate the amount of AAPH and CAF in bulk and in combined dosage forms.This method will be appropriate for the simultaneous determination of acetaminophen and caffeine in pharmaceutical forms: tablets, capsule and sachet.

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