[ISSN:2582-3663]

Canadian Journal of Biomedical Research and Technology

Can J Biomed Res & Tech

March 2020 Vol:3, Issue:1

H-Point Assay Method for Simultaneous Determination of Paracetamol and Caffeine in Panadol Extra Dosage Forms

Open Access **Research Article**

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Submission: March 15, 2020
Published: March 23, 2020

Abstract

A simple, specific, accurate and precise spectrophotometric method was settled for simultaneous determination of paracetamol and caffeine in pure form and in their pharmaceutical formulation commercially known as Panadol Extra®. H-Point assay has been used in simultaneous determination of both drugs without prior separation. H-Point assay method parameters were validated according to ICH guidelines in which accuracy, precision, repeatability and robustness were found in accepted limits. Advantages and disadvantages of H-point assay were discussed and statistical comparison between the proposed method and the reference method was performed.

Keywords: Spectrophotometric; Paracetamol; Caffeine; H-Point assay; ICH guidelines.

Introduction

Paracetamol (PAR); N-(4-Hydroxyphenyl) acetamide *(Figure 1)* is related to NSAID (non-steroidal anti-inflammatory drugs) which can act centrally and peripherally for treatment of non-inflammatory ailments in patients having gastric symptoms *[1]*. Caffeine (CAF) 1,3,7-trimethylxanthine *(Figure 1)* is a very important purine alkaloid which can act as a psycho stimulant by increasing alertness. Caffeine enters in many pharmaceutical preparations in combination with analgesic and antipyretic drugs as it increases their effect *[2]*.

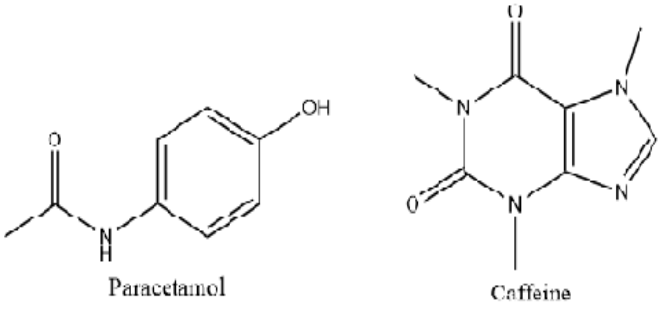

Figure 1: Chemical structures of paracetamol (PAR) and caffeine (CAF).

The literature showed that several methods were carried out for the analysis of PAR and CAF in their mixture form. PAR&CAF have been determined by spectrophotometric methods *[3-14]*, chromatographic methods *[15-23]*, voltammetric techniques *[24-26]*, NIR-chemometric method *[27]* and FI ultraviolet plus multioptosensing device technique *[28]*. No reported method for the estimation of this drug mixture by using H-point assay technique. As such, the aim of work is to develop a new spectrophotometric method which is accurate, fast and non-complicated for determination of PAR & CAF combination without the interference of their additives or their excipients in pharmaceutical formulations.

Experimental Apparatus

UV-visible spectrophotometer model V-630 (JASCO dual beam (Japan)) which is connected to an ACER compatible computer with the program (spectra manager II software) was used. The wavelength ranges were 200-400 nm at room temperature. Also, PASW statistics 18® software program was used for statistical analysis.

Materials and Reagents

Pure Standards

PAR was kindly provided by EIPICO (Egypt). Its purity was claimed to be as 99.50%. CAF was obtained from India (LABORT FINE CHEM), and its purity was 99.00%.

Pharmaceutical formulations

Panadol Extra® tablets have been purchased from the market (a

label claim: PAR 500 mg+CAF 65 mg) produced by Glaxo Smithkline, GSK, Egypt.

Solvents

Methanol (HPLC grade) was purchased from Germany (LiChrosolv, Merck KGaA). All of the measurements have been accomplished by using Methanol: Water 90: 10 (90% Methanol).

Standard Solutions

Standard stock solutions (1 mg/mL) of PAR and CAF have been prepared in 90% methanol. Working standard solution of PAR (40 µg/mL) and CAF (50 µg/mL) were prepared by further dilution with 90% methanol.

Laboratory Prepared Mixtures

Variable ratios of PAR & CAF have been performed by transferring accurate aliquots from the standard solutions to the volumetric flasks (10 mL) and then dilution was carried out with 90% methanol.

Procedures

Construction of Calibration Curves

For PAR: Working solutions equal to 4-22 µg/mL have been prepared by addition of accurate aliquots of (1, 1.50, 2, 2.50, 3, 3.50, 4, 4.50, 5, 5.50 mL) of PAR working standard solution (40 µg/mL) to 10 mL volumetric flasks followed by dilution with 90% methanol.

For CAF: Working solutions equal to 7.5-35 µg/mL have been prepared by adding accurate aliquots of (1, 1.50, 2, 2.50, 3, 3.50, 4, 4.50, 5, 6, 7 mL) of CAF working standard solution (50 µg/mL) to 10 mL volumetric flasks followed by dilution with 90% methanol.

Measurements of the absorption spectra have been carried out over the wavelengths (200-400 nm) at room temperature.

H-Point Assay Method

In the proposed method, two wavelengths were selected, 225 nm and 267 nm at which PAR exhibited the same absorptivity, in contrast to CAF that demonstrated sufficient difference in absorptivity at each of the selected wavelengths as shown in Fig. 2. Two calibration curves were constructed for PAR at 225 nm and 267 nm and its zero absorbance spectrum is showed in Fig. 3. On the other hand, CAF was the component, from which standard solutions with increased concentration could be added to a mixture of both components to determine their concentration by H-point standard addition method. Aliquots containing 7.50, 12.5, 17.5, 20, 22.5 µg/mL CAF were accurately added to the laboratory prepared mixture or pharmaceutical dosage form prepared solutions to be determined by this method. Curves of standard addition method were constructed where absorbances of solutions after CAF standard addition were represented on Y-axis while concentrations of the added CAF standard were represented on X-axis at the two selected wavelengths (225 nm and 267 nm). By plotting the absorbance versus added CAF concentration, two straight lines of different slopes and intercepts were obtained. As the absorbance value of PAR is constant at two selected wavelengths (225 nm and 267 nm), all the straight lines obtained at different wavelengths by applying the standard additions method will have common point. This point is known as the H point (Figure 4), the abscissa refers to CAF concentration (C CAF) alone and the Y coordinate is the absorbance of PAR (A PAR) alone in the corresponding mixture. The concentration of PAR (C PAR) in mixture was then determined by substitution in any of regression line equations at 225 nm and 267 nm.

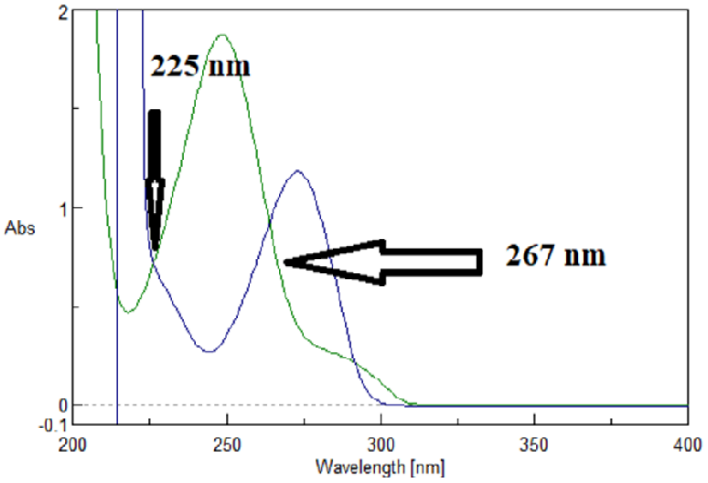

Figure 2: Zero absorption spectrum of 20 µg/mL PAR (green line) overlaid with 20 µg/mL CAF (blue line) showed that both 225 and 267 nm have the same absorbance for PAR spectrum.

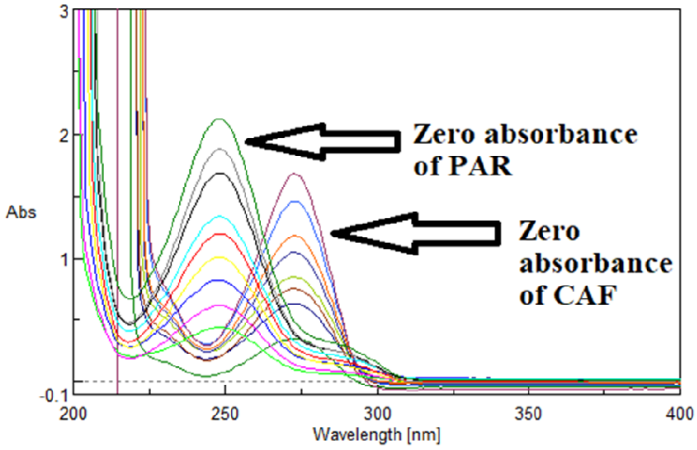

Figure 3: Zero absorption spectra of PAR and CAF

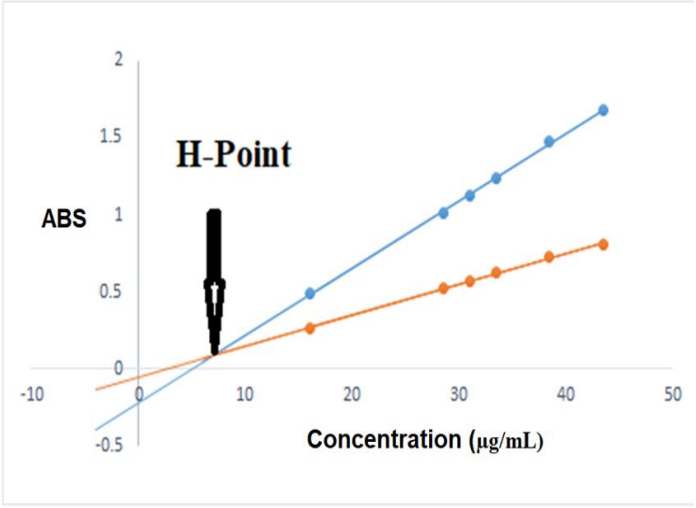

Figure 4: H-Point in the intercept of the two straight lines at 225nm & 267 nm after standard addition of CAF.

Analysis of Laboratory Prepared Mixtures

The spectra of mixtures were measured after preparation of variable ratios of the prepared laboratory mixtures then handled in the same conditions as described under each method.

Application to Pharmaceutical Formulation

Ten tablets of Panadol Extra® have been weighed and crushed then an amount equal to 50 mg PAR and 10 mg CAF in each tablet has been moved into a volumetric flask (50 mL) and diluted with 90% methanol as follow: First, 30 mL of 90% methanol have been added and sonicated then dilution has been carried out to the mark and filtered. Second, 10 mL of the dilution has been moved into a 100 mL volumetric flask to give a concentration equal to 100 µg/mL PAR and 20 µg/mL CAF. Third, any additional dilutions were carried out in volumetric flasks (10 mL) and handled in the same way as explained under each method.

Results and Discussion

Method Optimization

Two major problems were found during the analysis of PAR & CAF binary mixture; the first was the overlapped spectra between the absorptivity of both drugs, and the second, PAR, the main (major) constituent, had unfortunately very high absorbance, while CAF, the minor component, had low absorbance value. Intrinsically, sample enrichment technique *[29]* has been used in which the concentration of CAF (the minor component) in their dual mixtures has been increased to facilitate its determination. This was carried out by adding a fixed amount of standard CAF to each experiment when combined with PAR, then subtraction of its concentration before the calculation of the required CAF concentration. Sample enrichment technique has been used for solving the same problem in the analysis of other drug mixtures of different drug ratios [30-32].

H-Point Assay Method

225 and 267 nm absorbances were used far determination of PAR & CAF in presence of each other at the same wavelengths. The calibration curves revealed accepted linear relationships between concentrations and absorbance in a range of 4-22 µg/mL for PAR and 7.50-45 µg/mL for CAF with correlation coefficients ≥ 0.9990 for both drugs. The accuracy of the method illustrated accepted values with 100.42% ± 0.47 for PAR and 100.16% ± 1.23 for CAF. The results are detailed in Table 1. H-Point assay is very easy and simple as it depends on zero absorption spectra without the need of extra processing. On the other hand, it has two limitations; which are the need for some specific calculations to determine the values of H-Point in addition to requiring more time for performing the standard addition on each mixture.

Mixture	PAR & CAF			
Method Parameters	CAF		PAR	
Wave length (nm)	225	267	225	267
Linearity range (µg/mL) (n=3)	7.50-45	7.50-45	4-22	4-22
Intercept	-0.057	0.0395	0.0322	0.019452
Slope	0.0348	0.0409	0.0349	0.02717
Correlation coefficient (r)	0.9996	0.9996	0.999	0.9996
Accuracy (Mean ± SD)	100.16 ± 1.23		100.42 ± 0.47	
Precision (±%RSD)				
Repeatability	100.45 ± 0.99		98.84 ± 0.58	
Intermediate precision	100.74 ± 1.21		98.43 ± 0.79	
Specificity (Mean ± SD)	100.01 ± 0.80		100.32 ± 1.41	

Table 1: Assay parameters and validation results obtained by applying H-Point assay method.

Method validation

All methods were legalized as demonstrated by ICH guidelines *[33]*. The linear regression data for the calibration curves revealed good linear relationships *(Table 1)*. The accuracy has been assessed by analyzing the standard addition method where satisfactory results were achieved as shown in Tables 1,2. The specificity of this technique has been assessed by assaying the laboratory prepared mixtures of PAR & CAF within the linearity range and good results have been obtained *(Table 1)*. The intra- and inter-day precisions have been computed by the analysis of 3 different concentrations of the drugs 3 times on the same day in addition to 3 successive days *(Table 1)*.

	H-Point assay							
	CAF				PAR			
			Recovery%				Recovery%	
	Tablet Taken (µg/mL)	Standard Added (µg/mL)	Tablet	Added	Tablet Taken (µg/mL)	Standard Added (µg/mL)	Tablet	Added
	1.3	10	100.21	101.44	10	9	101.45	100.44
		11.3	100.74	100.04		10	100.54	100.88
		12	99.16	98.99		11	99.45	99.95
Mean			100.04	100.16			100.48	100.42
SD			0.8	1.23			1	0.47

Table 2: Analysis of the pharmaceutical Formulation (Panadol Extra® tablets) by applying H-Point assay method.

Application to Pharmaceutical Formulation

H-point assay method has been successfully used for determination of PAR&CAF in its pharmaceutical formulation (Panadol Extra® tablets). The results were acceptable in agreement with the labeled quantities. The standard addition method was used and revealed that no interference of the excipients was observed *(Table 2)*.

Statistical Analysis

Statistical comparison between the proposed technique and the reference method was done by One-way ANOVA method through utilizing PASW statistics 18® software program in which there was no significant difference between them *(Table 3)*.

Tablets	Drugs		Sum of Squares	df	Mean Square	F	Sig.
Panadol Extra® tablets	PAR	Between Groups	0.003	1	0.003	0.002	0.967
		Within Groups	5.854	4	1.464		
		Total	5.857	5			
	CAF	Between Groups	0.459	1	0.459	0.274	0.628
		Within Groups	6.697	4	1.674		
		Total	7.156	5			

Table 3: Results of the statistical comparison obtained by the proposed method and the reference method using One-way ANOVA.

Conclusion

H-Point assay method has been successfully applied for determination of paracetamol and caffeine in their binary mixtures and in their dosage form. This proposed method is simple, sensitive and accurate and could be used for regular analysis by using simple technology or instruments. By comparison with the previous reported methods, it was concluded that H-point assay method doesn't require extra processing but it needs more time and calculations. Statistical comparison revealed that there was no observed significant difference between the proposed method and the reference one.

References

1. Yehia AM, Abd El-Rahman MK (2014) Application of normalized spectra in resolving a challenging Orphenadrine and Paracetamol binary mixture. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy 138: 21-30.
2. Dash SS, Gummadi SN (2006) Catabolic pathways and biotechnological applications of microbial caffeine degradation. Biotechnology Letters 28: 1993-2002.
3. Antakli S, Nejem L, Dawoud M (2014) Spectrophotometric Determination of Paracetamol and Caffeine. Asian Journal of Chemistry 26: 7016-7020.
4. Tavallali H, Sheikhaei M (2009) Simultaneous kinetic determination of paracetamol and caffeine using Cu(II) neocuproine in presence of dodecyl sulfate by H-point standard addition method. Indian Journal of Chemistry Section A Inorganic, Physical, Theoretical and Analytical Chemistry 48: 812-816.
5. Moreira AB, Dias ILT, Neto GO, Zagatto EAG, Kubota LT (2006) Simultaneous spectrofluorimetric determination of paracetamol and caffeine in pharmaceutical preparations in solid-phase using partial least squares multivariate calibration. Analytical Letters 39: 349-360.
6. Hakan Aktaş A, Kitiş F (2014) Spectrophotometric Simultaneous Determination of Caffeine and Paracetamol in Commercial Pharmaceutical by Principal Component Regression, Partial Least Squares and Artificial Neural Networks Chemometric Methods). Croatica Chemica Acta 87: 69-74.
7. Nagendra P (2011) Spectrophotometric estimation of paracetamol in bulk and pharmaceutical formulations. E-Journal of Chemistry 8: 149-152.
8. Sapkota HP, Bhatta HP, Reddy MAK, Dangi NB, Babu CN, et al., (2015) Method Development and Validation of Dual Wavelength UV Spectrophotometric Method for Simultaneous Estimation of Paracetamol and Caffeine in Combined Dosage Form by Internal Standard Method. Asian Journal of Chemistry 27: 4666-4668.
9. Vichare V, Mujgond P, Tambe V, Dhole SN (2010) Simultaneous spectrophotometric determination of paracetamol and caffeine in tablet formulation. International Journal of PharmTech Research 2: 2512-2516.
10. Ortega-Barrales P, Padilla-Weigand R, Molina-Diaz A (2005) Simultaneous Determination of Paracetamol and Caffeine by Flow Injection-Solid Phase Spectrometry

Using C18 Silica Gel as a Sensing Support. Analytical Sciences 18: 1241-1246.

11. Delvadiya K, Kimbahune R, Kabra P, Sunil K, Patel P (2011) Spectrophotometric simultaneous analysis of paracetamol, propyphenazone and caffeine in tablet dosage forms. International Journal of Pharmacy and Pharmaceutical Sciences 3: 170-174.

12. Ahmida NHS, Abu-Naja MS, Doghman YSA (2009) Determination of paracetamol in tablet by difference spectrophotometric method. Asian Journal of Chemistry 21: 2233-2240.

13. Vidal AD, Barrales PO, Díaz AM (2003) Simultaneous Determination of Paracetamol, Caffeine and Propyphenazone in Pharmaceuticals by Means of a Single Flow-Through UV Multiparameter Sensor. Mikrochimica Acta 141: 157-163.

14. Moţ AC, Soponar F, Medvedovici A, Sârbu C (2010) Simultaneous spectrophotometric determination of aspirin, paracetamol, caffeine, and chlorphenamine from pharmaceutical formulations using multivariate regression methods. Analytical Letters 43: 804-813.

15. Delvadiya K, Kabra P, Kimbahune R, Patel N, Nargund LVG (2013) High-performance liquid chromatographic determination of paracetamol, propyphenazone, and caffeine in pharmaceutical formulations. Indian Journal of Pharmaceutical Education and Research 47: 65-72.

16. Muñoz RAA, Cunha RR, Torres LMFC, Santos WTP Dos, Chaves SC, et al., (2015) Simultaneous determination of caffeine, paracetamol, and ibuprofen in pharmaceutical formulations by high-performance liquid chromatography with UV detection and by capillary electrophoresis with conductivity detection. Journal of Separation Science 38: 1657-1662.

17. Radi M, Ramli Y, El Karbane M, Marzak S, Bougrin K, et al, (2016). Validation of a method for simultaneous determination of acetaminophen and caffeine by HPLC in different pharmaceutical forms: Tablet, capsule and sachet. Journal of Materials and Environmental Science 7: 4608-4613.

18. Redasani VK, Gorle AP, Surana SJ, Jain PS, Badhan RA (2012) Simultaneous determination of chlorpheniramine maleate, phenylephrine hydrochloride, paracetamol and caffeine in pharmaceutical preparation by RP-HPLC. Chemical Industry and Chemical Engineering Quarterly 19: 57-65.

19. Dewani AP, Chipade VD, Bakal RL, Chandewar AV, Kanungo SK, et al., (2012) RP-HPLC-DAD method for the determination of phenylepherine, paracetamol, caffeine and chlorpheniramine in bulk and marketed formulation. Arabian Journal of Chemistry 7: 811-816.

20. Sharma S, Sharma MC, Sharma R, Sharma AD (2011) High Performance Liquid Chromatographic Assay method for the determination of Paracetamol and Caffeine in Tablet Formulation-in vitro dissolution studies. Journal of Pharmacy Research 4: 1559-1561.

21. Altun ML (2002) HPLC method for the analysis of paracetamol, caffeine and dipyrone. Turkish Journal of Chemistry 26: 521-528.

22. Emre D, Özaltin N (2007) Simultaneous determination of paracetamol, caffeine and propyphenazone in ternary mixtures by micellar electrokinetic capillary chromatography). Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 847: 126-132.

23. Narayanan VL, Austin A (2016) Determination of Acetaminophen and Caffeine using reverse phase liquid (RP-LC) chromatographic technique. Journal of Research in Pharmaceutical Sciences 3: 5-10.

24. Lau OW, Luk SF, Cheung YM (1989) Simultaneous determination of ascorbic acid, caffeine and paracetamol in drug formulations by differential-pulse voltammetry using a glassy carbon electrode. The Analyst 1047-1051.

25. Yiğit A, Yardim Y, Şentürk Z (2016) Voltammetric Sensor Based on Boron-Doped Diamond Electrode for Simultaneous Determination of Paracetamol, Caffeine, and Aspirin in Pharmaceutical Formulations. IEEE Sensors Journal 16: 1674-1680.

26. Saciloto RT, Cervini P, Cavalheiro É (2013) Simultaneous Voltammetric Determination of Acetaminophen and Caffeine at a Graphite and Polyurethane Screen-Printed Composite Electrode. Journal of the Brazilian Chemical Society 24: 1461-1468.

27. Muntean DM, Alecu C, Tomuta I (2017) Simultaneous Quantification of Paracetamol and Caffeine in Powder Blends for Tableting by NIR-Chemometry. Journal of Spectroscopy 2017: 1-8.

28. Ruiz Medina A, Fernandez De Córdova ML, Molina-Diaz A (1999) Simultaneous determination of paracetamol, caffeine and acetylsalicylic acid by means of a FI ultraviolet pls multioptosensing device. Journal of Pharmaceutical and Biomedical Analysis 21: 983-992.

29. Lotfy HM, Tawakkol SM, Fahmy NM, Shehata MA (2014) Successive spectrophotometric resolution as a novel technique for the analysis of ternary mixtures of pharmaceuticals. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy 121: 313-323.

30. Moussa BA, Mahrouse MA, Fawzy MG (2018) Different resolution techniques for management of overlapped spectra: Application for the determination of novel co-formulated hypoglycemic drugs in their combined pharmaceutical dosage form. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy 205: 235-242.

31. Lotfy HM, Mohamed D, Mowaka S (2015) A comparative study of smart spectrophotometric methods for simultaneous determination of sitagliptin phosphate and metformin hydrochloride in their binary mixture.

Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy 149: 441-451.

32. Sebaiy MM, El-adl SM, Mattar AA (2020) Different techniques for overlapped UV spectra resolution of some co-administered drugs with paracetamol in their combined pharmaceutical dosage forms. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 224: 117429.

33. ICH (1994) Validation of Analytical Procedure: Text and Methodology Q2 (R1)). ICH Steering Committee 2005: 13.