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Spectrum subtraction method for simultaneous determination of acetaminophen and caffeine in panadol extra dosage forms

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RESEARCH ARTICLE

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ABSTRACT

A simple, specific, accurate and precise spectrophotometric method was established for simultaneous determination of acetaminophen and caffeine in pure form and in their pharmaceutical formulation commercially known as Panadol Extra®. Spectrum subtraction has been used in simultaneous determination of both drugs without prior separation. Spectrum subtraction method parameters were validated according to ICH guidelines in which accuracy, precision, repeatability and robustness were found in accepted limits (98-102%). The linearity range was 7.5-45 µg/mL for caffeine and 4-22 µg/mL for acetaminophen with correlation coefficients ≥0.9990 for both drugs. Advantages and disadvantages of spectrum subtraction were discussed and statistical comparison between the proposed method and the reference one was performed.

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1. Introduction

Acetaminophen (APAP); *N*-(4-hydroxyphenyl)acetamide (Figure 1) is related to non-steroidal anti-inflammatory drugs (NSAID) which can act centrally and peripherally for treatment of non-inflammatory ailments in patients having gastric problems [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].

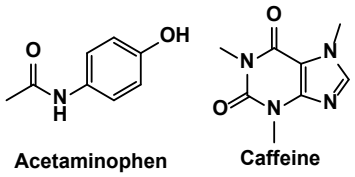

Figure 1. Chemical structures of acetaminophen (APAP) and caffeine (CAF).

The literature showed that several methods were carried out for the analysis of APAP and CAF in their mixture form. APAP and CAF have been determined by spectrophotometric methods [3-14], chromatographic methods [15-23], voltammetric techniques [24-26], Near Infrared (NIR)-chemometric method [27] and Flow-through (FI) ultraviolet plus multiopto-sensing device technique [28].

To the best of our knowledge, no method for the estimation of this drug mixture by using spectrum subtraction technique was yet reported. As such, the aim of the current work is to develop a new spectrophotometric method which is accurate, fast and non-complicated for determination of APAP and CAF combination without the interference of their additives or their excipients in pharmaceutical formulations.

2. Experimental

2.1. 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.

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2.2. Materials and reagents

2.2.1. Pure standards

APAP 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%.

2.2.2. Pharmaceutical formulations

Panadol Extra® tablets have been purchased from the market (a label claim: APAP 500 mg + CAF 65 mg) produced by Glaxo Smithkline, GSK, Egypt.

2.2.3. Solvents

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

2.2.4. Standard solutions

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

2.2.5. Laboratory prepared mixtures

Variable ratios of APAP and 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.

2.3. Procedures

2.3.1. Construction of calibration curves

For APAP: Working solutions equal to 4-22 µg/mL have been prepared by addition of accurate aliquots of 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50 mL of APAP 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 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 6.00, 7.00 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.

2.3.2. 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.

2.3.3. Application to pharmaceutical formulation

Ten tablets of Panadol Extra® have been weighed and crushed then an amount equal to 50 mg APAP 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 APAP and 13 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.

2.4. Theory/calculation

2.4.1. Spectrum subtraction method

The method relies on subtracting the spectrum of Y from the spectrum of the mixture (X + Y), therefore we can obtain the zero absorption spectrum of X again. This can be summarized as the following:

$$(X + Y) - Y = X \quad (1)$$

The concentration of X is calculated from the corresponding regression equation obtained by plotting the absorbance values of the zero order absorption spectra of X at its λ_{max} against the corresponding concentrations. Zero absorption spectra of APAP and CAF can be recovered from their mixture through spectrum subtraction of CAF and APAP, respectively (Figure 2). Zero absorption spectra of CAF and APAP are shown in Figure 3.

3. Results and discussion

3.1. Method optimization

Two major problems were found during the analysis of APAP and CAF binary mixture; the first was the overlapped spectra between the absorptivity of both drugs, and the second, APAP, 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 APAP, then subtraction of its concentration before the calculation of the required CAF concentration. Sample enrichment technique has been used also for solving the same problem in the analysis of other drug mixtures of different drug ratios [30-32].

3.2. Spectrum subtraction method

248 and 273 nm absorbances were used for determination of APAP and CAF in presence of each other, respectively. The calibration curves revealed accepted linear relationships between concentrations and absorbance in a range of 4.00-22.00 µg/mL for APAP and 7.50-45.00 µg/mL for CAF with correlation coefficients ≥ 0.9990 for both drugs. The accuracy of the method illustrated accepted values with 101.40% ± 0.45 for APAP and 99.28% ± 1.05 for CAF. The results are detailed in Table 1.

Spectrum subtraction is very easy and simple as it depends on zero absorption spectra without the need of extra processing. It is having few steps to get the zero order spectra of the desired drug but it suffers from noise interference while acquiring the desired drug concentration by subtraction.

3.3. Method validation

All methods were legalized as demonstrated by International Council for Harmonisation (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 and 2. The specificity of this technique has been assessed by assaying the laboratory prepared mixtures of APAP and CAF within the linearity range and good results have been obtained (Table 1).

Table 1. Assay parameters and validation results obtained by applying Spectrum Subtraction method.

Method parameters	CAF	APAP
Wavelength (nm)	273	248
Linearity range (µg/mL) (n=3)	7.50-45.00	4.00-22.00
Intercept	0.0010	0.0800
Slope	0.0481	0.0911
Correlation coefficient (r)	0.9990	0.9990
Accuracy (Mean±SD)	99.28±1.05	101.40±0.45
Precision (±%RSD)		
Repeatability	100.98±0.39	99.11±0.12
Intermediate precision	100.24±0.19	99.81±0.48
Specificity (Mean±SD)	100.20±1.44	99.09±1.58

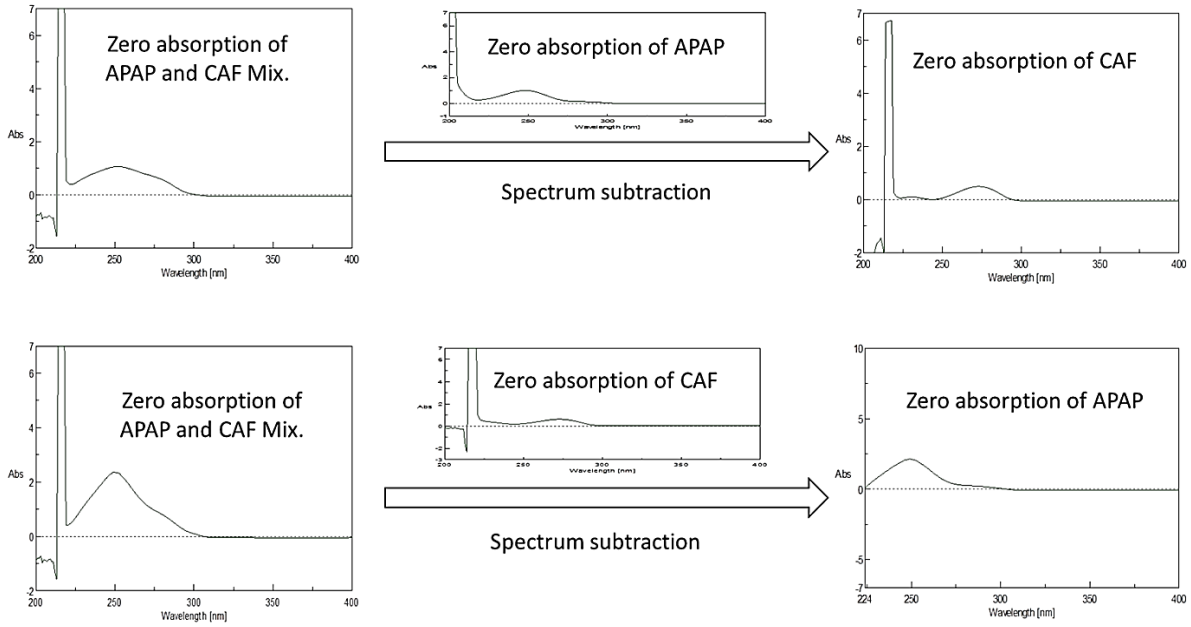

Figure 2. Spectrum subtraction of CAF and APAP from their mixture resulting in absorption spectra of APAP and CAF, respectively.

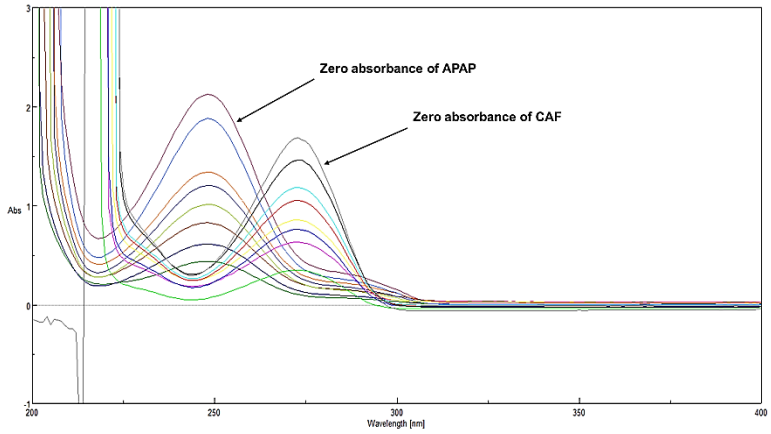

Figure 3. Zero absorption spectra of APAP and CAF.

The intra- and inter-day precisions have been computed through the analysis of three different concentrations of the drugs three times on the same day in addition to 3 successive days (Table 1).

3.4. Application to pharmaceutical formulation

Spectrum subtraction method has been successfully used for determination of APAP and 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).

3.5. Statistical analysis

Statistical comparison between the proposed technique and the reference one [3] 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).

Table 2. Analysis of the pharmaceutical formulation (Panadol Extra® tablets) by applying spectrum subtraction method.

H-Point assay							
CAF		**Recovery %**		**APAP**		**Recovery %**	
Tablet taken (µg/mL)	**Standard added (µg/mL)**	**Tablet**	**Added**	**Tablet taken (µg/mL)**	**Standard added (µg/mL)**	**Tablet**	**Added**
1.30	10.00	100.40	98.84	10.00	9.00	99.15	101.11
	11.30	99.55	100.47		10.00	100.48	101.19
	12.00	100.87	98.52		11.00	98.09	101.92
Mean		100.27	99.28	**Mean**		99.24	101.40
SD		0.67	1.05	**SD**		1.20	0.45

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

Tablets	**Drugs**	**Mean ± SD**	**Reference (Mean ± SD)**		**Sum of squares**	**df**	**Mean square**	**F**	**Sigma**
Panadol Extra ® tablets	APAP	99.24±1.20	100.03±1.53	Between groups	0.013	1	0.013	0.008	0.934
				Within groups	6.730	4	1.682		
				Total	6.743	5			
	CAF			Between groups	3.139	1	3.139	4.932	0.091
		100.27±0.67	100.11±1.61	Within groups	2.546	4	0.637		
				Total	5.685	5			

4. Conclusion

Spectrum subtraction method has been successfully applied for determination of acetaminophen 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 spectrum subtraction method is very simple and doesn't require extra processing. Statistical comparison revealed that there was no observed significant difference between the proposed method and the reference one.

Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
Author contributions: All authors contributed equally to this work.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

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