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DERIVATIVE SPECTROPHOTOMETRIC DETERMINATION OF QUININE, ADENINE AND METOCLOPRAMIDE HYDROCHLORIDE IN THEIR BINARY MIXTURE USING ZERO–CROSSING TECHNIQUE

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ABSTRACT. A first–derivative spectrophotometric procedure has been applied for the simultaneous quantification of quinine–adenine or quinine–metoclopramide hydrochloride in their mixes using "zero–crossing" measuring approach. The first–derivative spectra of quinine and adenine allowed the determination of quinine (1.5–17.9 µg/mL) in the presence of 4.3 µg/mL of adenine, and adenine (5.4–27.0 µg/mL) in presence of 9.0 µg/mL of quinine. In the binary mixture of quinine–metoclopramide hydrochloride, the first–derivative spectra of quinine and metoclopramide hydrochloride allowed the determination of quinine (11.95–95.62 µg/mL) in presence of 5.4 µg/mL of metoclopramide hydrochloride, and metoclopramide hydrochloride (1.34–21.52 µg/mL) in existence of 29.88 µg/mL of quinine. A statistical scrutiny of the provided practical data was used to perform a critical assessment of the recommended approaches.

KEY WORDS: Quinine, adenine, Metoclopramide hydrochloride, Binary mixture, Simultaneous determination, Derivative spectrophotometric

INTRODUCTION

Quinine (QN), cinchonan-9-ol,6'methoxy,(8α,9R)hydrochloride (1:1) salt dihydrate, is a member of cinchona alkaloids group, together with quinidine, cinchonine and cinchonidine. The structure of QN is mentioned in Figure 1 [1]. These compounds were characterized as naturally occurring and commonly encountered in the cinchona tree bark which growing in South America [1]. As well as QN is the original antimalarial agent, it is used as antibacterial, antipyretic, moderate oxytocic, local anesthetic, cardiovascular stimulant, and analgesic characteristics, and is also used to prevent cardiac arrhythmias [2]. Furthermore, compared to its descendent pharmaceuticals, hydroxychloroquine and chloroquine, QN demonstrated reduced cytotoxicity and antiviral efficacy against SARS-CoV-2 [3]. Also, it was concluded that QN can be improved as a COVID–19 therapies [4]. Prolonged administration of QN may produce vigorous side effects such as headache, deafness, vasodilation, perspiring, nauseousness, tinnitus, lightheadedness, indistinct visibility, skin rashes and digestive upsets [5]. In addition to QN medical application, and thanks to its distinctive bitter taste and aroma, it is very popular food additive and frequently added to tonic water which is a non-alcoholic carbonated beverage [6].

Adenine (AN), 6-amino-3H-purine (Figure 1) [7], belongs to purines which is a type of nucleoside that can have a variety of physiological effects, including central nervous system stimulation, gastric acid secretion and diuresis [7]. Purines also been associated in heart disease, cancer, kidney failure and asthma. Moreover, nadide (which is a dinucleotide of adenine and nicotinamide) may have a role to play in avoiding COVID-19 [8, 9].

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Metoclopramide hydrochloride (MCP) known as monohydrate of 4-amino-5-chloro-N-[(2-diethylamino)ethyl]-2-methoxy benzamide hydrochloride (Figure 1) [10]. It's a substituted benzamide that's often used as an anti-emetic to treat nausea and vomiting, as well as to stimulate the motility of the upper gastrointestinal tract [10]. The lower esophageal sphincter is enforced by MCP, which reduces stomach acid reflux. MCP also speeds up the passage of solid and liquid meals out from stomach into the intestines. Meals that are quickly digested assist to prevent the reflux of stomach acid and other substances into the esophagus [11]. Conjunction between MCP and dexamethasone was utilized to cure intense queasiness and puking caused by some cancer treatments [12].

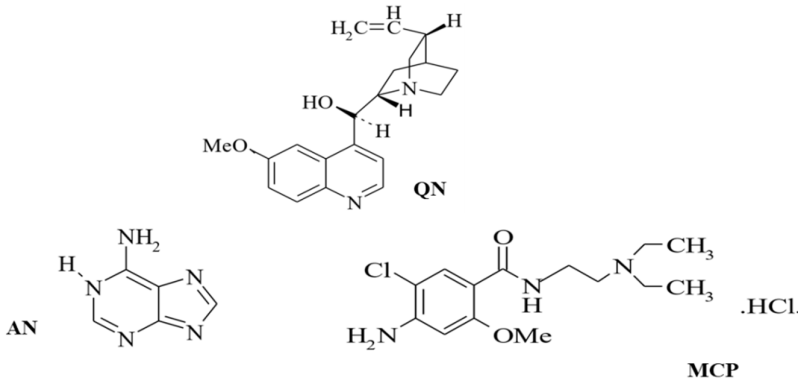

Figure 1. Structures for QN, AN and MCP.

Analytical chemistry plays an essential role in pharmaceutical industries development of to ensure products quality offered in the market, as well as ensuring environmental, economic and social sustainability. Various analytical approaches were used for the quantification of studied drugs in current article. These methods include chromatographic [13–19], electrochemical [20–30] and spectrophotometric methods [31–39].

Analytical selectivity is one of the most crucial requirements of analytical methods. Some methods' low selectivity makes them inappropriate for applications without using masking agents or a preparatory step to separate analyte from complex matrices, resulting in a prolonged time-consuming analysis. In most cases, spectrophotometric quantification of chemical preparations in pharmaceutical products face a great challenge due to the occurred overlapping of their spectra or their related species spectra with that of chromogenic reagents [40,41]. Derivative spectrophotometry techniques were utilized overcome problems joined the determination of an individual biological molecules and pharmaceuticals from overlapped spectra [42–47]. Moreover, such techniques are cheap, fast, accurate, and of precise analytical procedures, which make it of great demand in analytical and quality control laboratories utilized in pharmaceutical industry improvement. Herein, we present an initiative that can be used for the same purpose. This initiative represented in simultaneous spectrophotometric determination of QN in its binary mixtures with AN or MCP without need for pre-treatment of the samples. The approach was accurate and precise enough to allow for quick and cost-effective assay of QN, AN and MCP at very lower limit of detection (LOD) and quantification (LOQ). Also, because less energy is used during the process, fewer solvents and reagents are used, and less waste is generated, the utilized procedure is more environmentally friendly and greener. Herein and based on obtained results of presented procedure, we hope to support sustainability in pharmaceutical industry.

EXPERIMENTAL

Chemicals and reagents

All utilized chemicals were analytical reagent grade and were used without any purification steps. Both of quinine and adenine where purchased from Aldrich-Sigma Chemical Company, St. Louis, Missouri, USA while metoclopramide hydrochloride and hydrochloric acid were purchased from Merck, Germany. All chemicals were authentic samples and were used without any purification.

Instruments and apparatus

All absorption measurements were performed with a Perkin-Elmer Lambda 3B double–beam UV-Visible programmable spectrophotometer (Model C618-0437, USA) with 1 cm quartz cell and PC running the spectrophotometric software PECSS. The first-derivative was obtained using the PECSS Perkin-Elmer software program with a 1.0 nm slit width, 120 nm min^{-1} scan speed, and 1.0 nm wavelength interval. VWR Scientific Products Model 2000, USA, was used to take all of the pH readings. Standard buffer pH ≈ 4.01, 7.00 and 9.00 were used to calibrate the electrode.
Procedures

Drug solutions preparation

Quinine (QN). Typical standard solution of QN (10^{-2} M) was prepared by dissolution 0.3735 g of pure QN in bi-distilled water (at 20 °C, 1.0 g of QN were dissolved per 23 mL bi-distilled water), and completed to 100-mL using the same solvent. The operational solution of QN was daily prepared by consecutive dilution using bi-distilled water.

Adenine (AN). Stock solution of AN (10^{-2} M) was set by dissolving 0.135 g of the pure drug in 0.1 M HCl and diluted with the same solvent to 100-mL. Daily, the AN working standard solution was made by serial dilution of the stock standard solution in the same solvent.

Metoclopramide hydrochloride (MCP). Stock standard solution of MCP (10^{-2} M) was prepared by dissolution 0.3363 g of the real MCP in bi-distilled water in 100-mL volumetric flask and the volume was completed with the same solvents (at 25 °C 48.0 g of MCP were dissolved in 100-mL bi-distilled water). MCP working solutions were made every day by sequentially diluting the stock standard solution with bi-distilled water.

Simultaneous derivative spectrophotometric determination of QN and AN in their binary mixtures

Introduce a sample or standard solution containing 1.5-17.9 µg/mL of QN and 5.4-27.0 µg/mL of AN into 25-mL volumetric flask. Add 5 mL 0.1 M HCl and complete the volume with bi-distilled water. The derivative spectra from 325 to 210 nm were recorded against the blank reagent. First-derivative absolute values for QN and AN, were measured at 260 and 244 nm, respectively.

Simultaneous derivative spectrophotometric determination of QN and MCP in their binary mixtures

Suitable aliquots containing 12.0-95.6 µg/mL of QN and 1.3-21.5 µg/mL of MCP and 5 mL 0.1 M HCl were introduced into 25-mL volumetric flask. With bi-distilled water, the volume was brought up to the required level. Against a reagent blank, derivative spectra were measured from 434 to 260 nm. For the quantification of QN and MCP, the first-derivative values were recorded at 276 and 315 nm, respectively.

RESULTS AND DISCUSSION

Derivative spectrophotometric measurements of QN and AN in binary mixture

The absorption spectra of individual AN and QN, as well as their combination in 0.02 M HCl, are shown in Figure 2 (A). Individual AN and QN absorption spectra are shown in curves 1 and 2, respectively. Their absorption spectra have maxima of 260 nm for AN, 344, 316, and 244 nm for QN. Curve 3 depicts the absorption spectra of an AN and QN combination.

Because the spectral bands of different chemicals overlap, determining AN and QN in their mixtures using standard spectrophotometry is problematic. The above study was carried out using 5.40 µg/mL of AN and 8.96 µg/mL of QN in 0.02 M HCl.

Simultaneous analysis of binary mixtures was investigated, were the total absorbance of the mixture at the two wavelengths of maximal absorption were recorded followed by using simultaneous equations. However, low accuracy and repeatability results were achieved because the absorption spectra of the two compounds were not effectively separated. As a consequence, it was revealed that solving the problem of tightly overlapped spectra utilizing the derivative spectra of the combination of both substances was more basic and simpler.

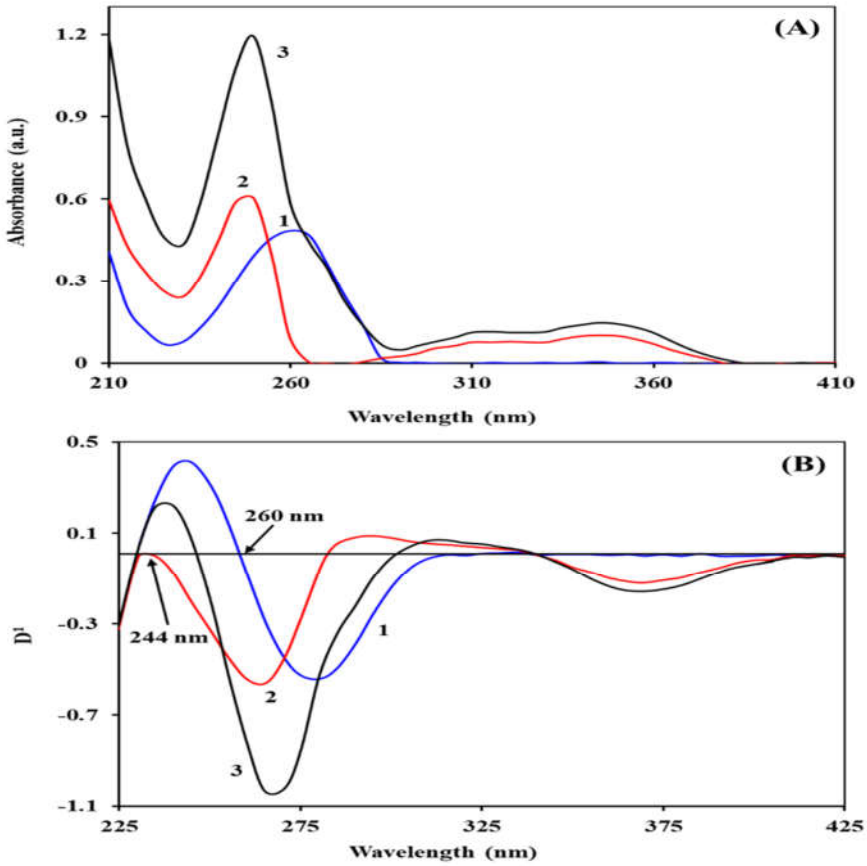

Figure 2. (A) Zero-order and (B) first order derivative absorption spectra of: (1) 5.4 µg/mL of AN, (2) 8.96 µg/mL of QN and (3) mix of both AN and QN.

A study of the first-, second-, third- and fourth-derivative spectra of AN and QN mixtures proved that the first-derivative spectra gave results of highest accuracy, good reproducibility and lower detection limits. Figure 2 (B) shows the first-derivative spectra of the compounds shown in Figure 2 (A). The first-derivative spectra of a combination of both substances (Figure 2 (B) curve 3) are clearly not resolved enough to provide two separate peaks. As a result, for resolving the combination of the selected chemicals, the zero-crossing measurement approach was used.

For the calibration graphs, the zero-crossing measurement approach was employed to choose wavelengths at which the data were proportionate to the AN and QN concentrations. This method is used to determine the absolute value of the total derivative spectrum at wavelength value, which corresponds to the zero-crossing point in the spectrum of the interfering component. As a result, measurements of the value of a mixture's derivative made at the zero-crossing point of one of the two substances' derivative spectrum will be a function exclusively of the other component concentration. The zero-crossing wavelengths of AN and QN occur at 260 and 244 nm, respectively, in Figure 2 (B).

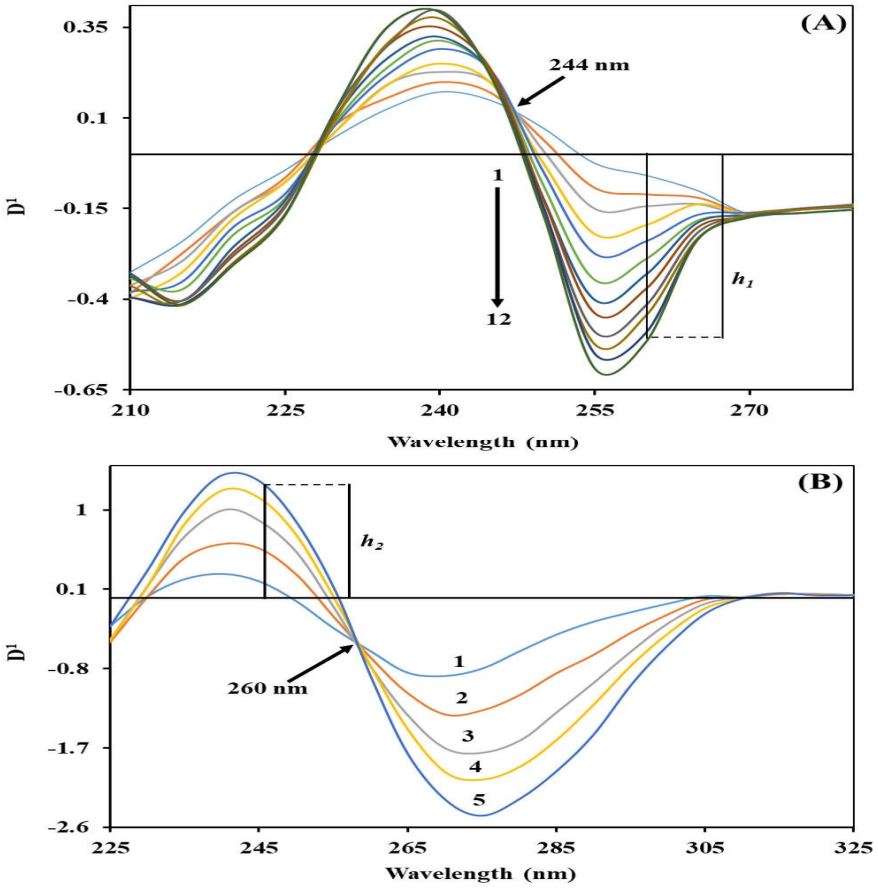

Figure 3. First-derivative absorption spectra of: (A) increasing QN concentration in presence of 4.3 µg/mL AN and (B) increasing AN concentration in existence of 9.0 µg/mL QN.

Figure 3 (A) illustrates a variety of first–derivative spectra for mixtures of 4.3 µg/mL of AN plus successive escalating QN concentrations ranging from 1.5 to 17.9 µg/mL. A series of first-derivative spectra of the mixtures containing 9.0 µg/mL of QN and variable AN concentration in the range 5.4-27.0 µg/mL are given in Figure 3 (B). The height h1 and h2 in the first–derivative spectrum of mixtures Figure 3, equivalent to derivative values taken at wavelength 260 nm (zero-crossing wavelength of the first-derivative spectrum of AN) and 244 nm (zero-crossing wavelength of the first-derivative spectrum of QN) were proportionate to the concentrations of QN and AN, respectively.

Derivative spectrophotometric measurements of QN and MCP in binary mixture

Figure 4 (A) shows the absorption spectra (zero-order) of MCP (curve 1), with maximum at 304 and 276 nm, QN (curve 2), with maximum at 345, 315 and 247 nm and a mixture of QN and MCP (curve 3). The absorption spectra of the compounds were gained using 5.4 µg/mL MCP and 8.9 µg/mL QN in 0.02 M HCl.

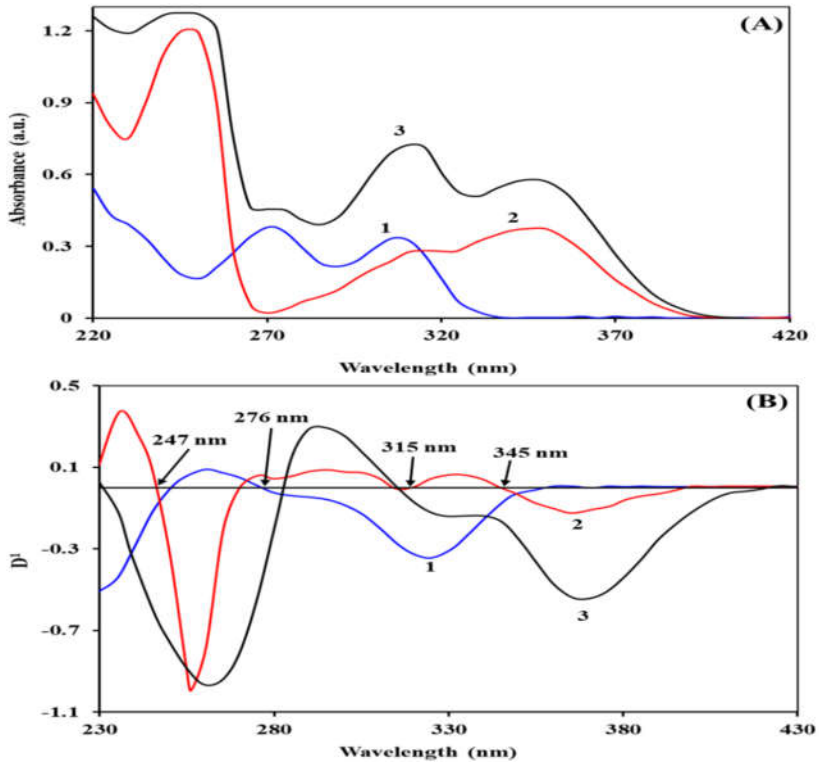

Figure 4. (A) Zero-order and (B) first order derivative absorption spectra of: (1) 5.4 µg/mL of MCP, (2) 29.88 µg/mL of QN and (3) mix of both MCP and QN.

As shown in Figure 4 (A), pure drug zero-order spectra were seen to overlap, making simultaneous detection problematic. As can be observed, the absorption spectra of MCP and QN are remarkably similar, making it impossible to distinguish between the two medications using direct absorbance measurements.

The first-derivative spectra of each pure drug, on the other hand, were discovered to have zero-crossing spots (Figure 4 (B)), which helped with simultaneous quantification. The first-derivative spectra's shape was sufficient for identifying MCP in the presence of QN and vice versa. MCP concentration was calculated by measuring its D1 amplitude at QN's zero crossing point (at 315 nm), whereas QN was quantified by measuring its D1 at MCP's zero crossing point (at 276 nm).

A set of first-derivative spectra was gained for mixtures comprising 5.4 µg/mL MCP and several QN concentrations (11.95-95.62 µg/mL) are given in Figure 5 (A). Figure 5 (B) shows a set of first-derivative spectra obtained for mixtures containing 29.88 µg/mL QN and variable MCP concentrations (1.34-21.52 µg/mL). MCP interference was avoided by taking into account the derivative value for a combination of QN and MCP at 276 nm, and QN concentration was calculated. The interference caused by QN was avoided by mensuration the derivative value for a combination of QN and MCP at 315 nm, and MCP concentration can be computed.

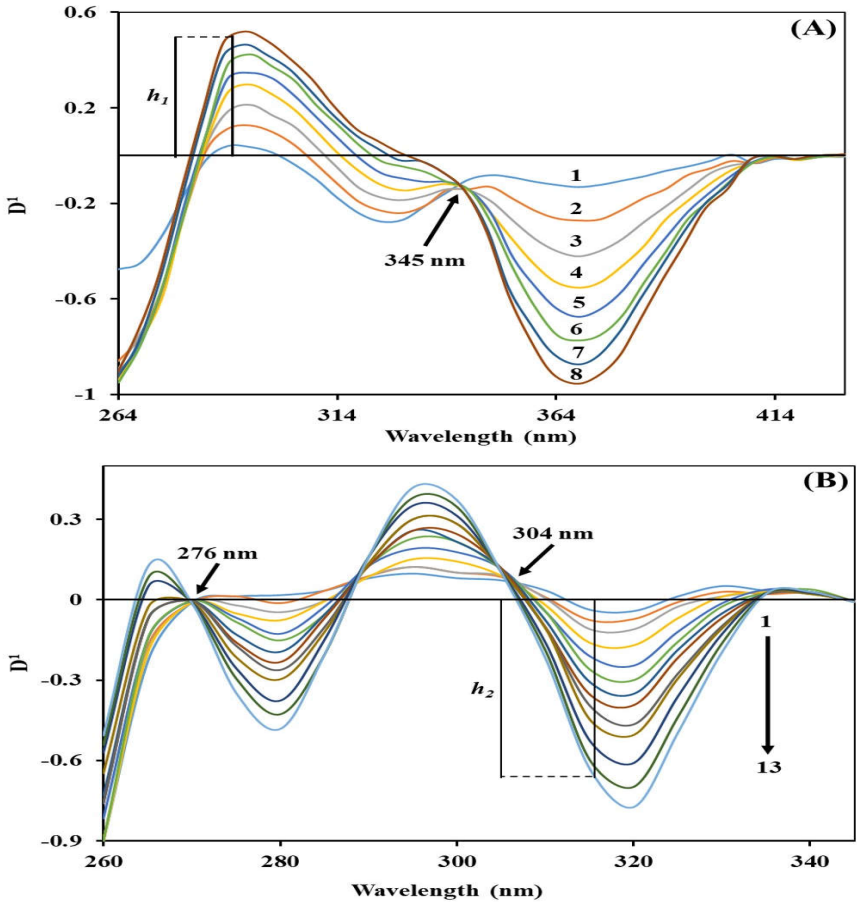

Figure 5. First-derivative absorption spectra of: (A) increasing QN concentration in existence of 5.4 µg/mL MCP and (B) increasing MCP concentration in existence of 29.88 µg/mL QN.

Calibration graphs, analytical parameters and characteristic statistical data

To obtain a linear calibration graphs passing through the origin for AN and QN determination, the recommended procedures were followed. The simultaneous quantification calibration graphs of AN and QN were constructed by graphing the first-derivative value (h) vs AN or QN concentration using the zero-crossing approach. The calibration graphs for simultaneously quantification of AN and QN in their binary mixes are shown in Figure 6. The first-derivative-concentration relationship achieved by suggested procedure was linear through a range of 5.4-27.0 µg/mL of AN in the existence of 8.96 µg/mL of QN and 1.50-17.90 µg/mL of QN in the existence of 4.32 µg/mL of AN.

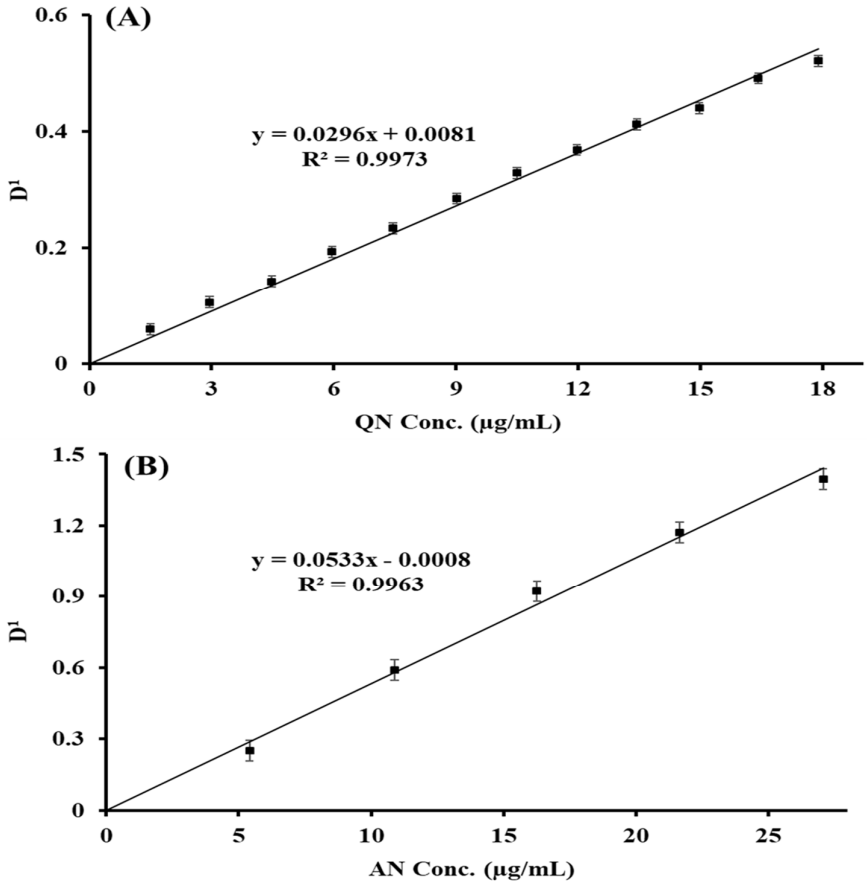

Figure 6. Calibration graph obtained for: (A) determination of QN in presence of 4.32 µg/mL AN and (B) determination of AN in presence of 8.96 µg/mL of QN.

The obtained relationships result in straight lines passing through the origin, suggesting that the derivative values of the two components are alternately independent. Using 10 identical mixtures containing 6.8 µg/mL of AN and 5.9 µg/mL of QN, the accuracy of the first-derivative approach for the simultaneous measurement of AN and QN was computed [48]. For a combination comprising 6.8 µg/mL of AN and 5.6 µg/mL of QN, the relative standard deviation was 0.21% and 0.28%, respectively.

The analytical parameters of the proposed method and statistical data for calibration graphs are illustrated in Table 1. The high correlation coefficient values as well as intercept on y-axis is near to nil indicates that all calibration graphs should a decent linearity and comply to Beer's law. Furthermore, the results illustrated in Figure 6 as well as Table 1 imply that the first-derivative zero-crossing technique achieved a reasonable resolution of the obtained two overlapped spectra.

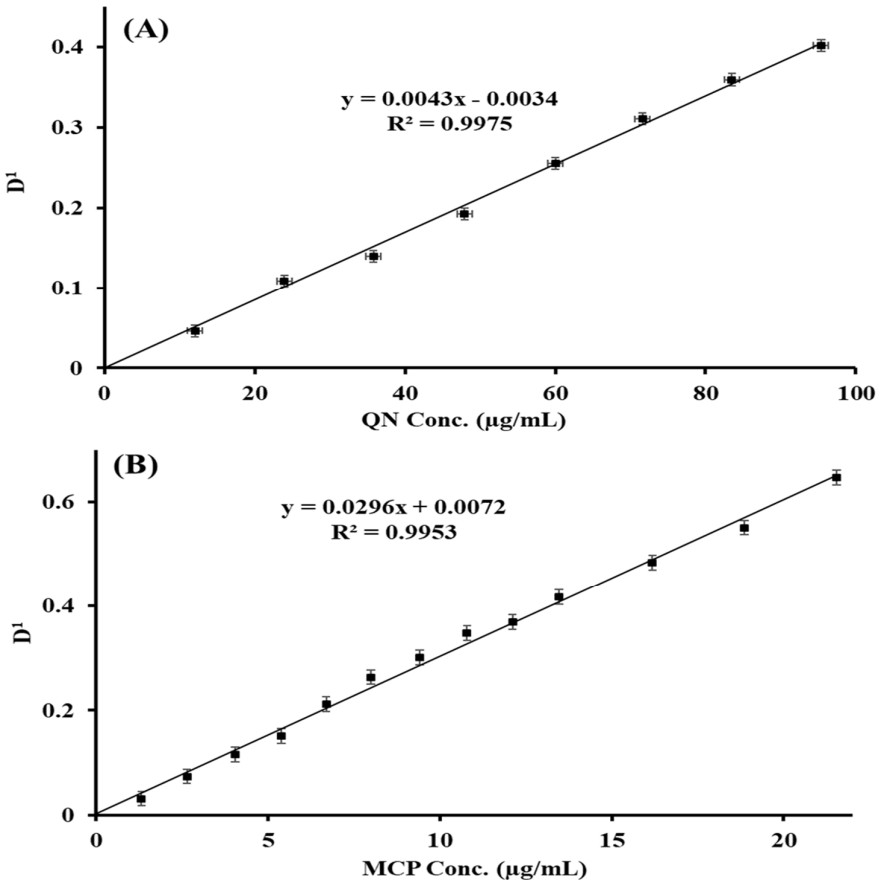

Figure 7. (A) First derivative-QN concentration relationship in presence of 5.4 µg/mL MCP and (B) first derivative-MCP concentration relationship in presence of 29.88 µg/mL QN.

The calibration graphs for the simultaneous quantification of MCP and QN in existence of each other were obtained by graphing the value of first-derivative measurement by zero–crossing technique against the MCP or QN concentration. Figure 7 illustrate the first–derivative–

concentration relationship of MCP and QN compounds, respectively. The linear dynamic ranges for quantification of MCP and QN simultaneously were 1.30–21.50 µg/mL MCP in the existence of 29.88 µg/mL of QN and 12.00-95.37 µg/mL QN in the presence of 5.4 µg/mL of MCP. The relative standard deviation (n = 10) for mixture consists of 5.8 µg/mL MCP and 15.4 µg/mL of QN was 0.17 and 0.45, respectively. Both of lower limit of detection (LOD) and quantification (LOQ) were calculated using the following equations: LOD = 3.3σ/b and LOQ = 10σ/b, where σ is the standard deviation of the intercept and b is the slope of the calibration curve [48]. Statistical variables for obtained calibration graphs are provided in Table 1. Figure 7 as well as Table 1 indicates that decent resolution of the obtained two overlapped spectra is possible utilizing zero–crossing first–derivative spectrophotometry.

Table 1. Statistical analysis for QN simultaneous quantification in binary mixes with AN or MCP.

Mixture	Drug	Regression equation*	Linearity range (µg mL^{-1})	R^2	Intercept ± SD	Slope ± SD	RSD	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
QN–AN	QN	$D^1 = 0.0296\ C + 8.1\times10^{-3}$	1.50–17.90	0.9973	$8.1\times10^{-3} \pm 4.94\times10^{-3}$	$2.96\times10^{-2} \pm 4.0\times10^{-4}$	3.07	0.50	1.67
	AN	$D^1 = 0.0533\ C - 8.0\times10^{-4}$	5.40–27.00	0.9963	$-8.0\times10^{-4} \pm 2.65\times10^{-2}$	$5.33\times10^{-2} \pm 1.9\times10^{-3}$	4.67	1.49	4.97
QN–MCP	QN	$D^1 = 0.0043\ C - 3.4\times10^{-3}$	12.00–95.37	0.9975	$-3.4\times10^{-3} \pm 4.6\times10^{-3}$	$4.3\times10^{-3} \pm 8.1\times10^{-5}$	3.34	3.22	10.73
	MCP	$D^1 = 0.0296\ C + 7.2\times10^{-3}$	1.30–21.50	0.9953	$7.2\times10^{-3} \pm 6.62\times10^{-3}$	$2.96\times10^{-2} \pm 5.87\times10^{-4}$	4.62	0.67	2.24

*C = µg mL^{-1}, R^2 = correlation coefficient.

Literature reviews show several methods for the determination of QN, AN and MCP. Some of these methods are chromatographic [13–19] and electrochemical [20–30] methods, which are expensive and require many extraction and pretreatment steps before taking measurements. Regarding spectrophotometric methods [31–39], results obtained from our procedure are much better or even very close to those obtained from previous spectrophotometric methods. Moreover, our suggested procedure is characterized as the lowest cost and simplest procedure for the determination of suggested compounds. Table 2 shows a comparison between results obtained from our procedure and those obtained from previous spectrophotometric methods.

Table 2. Data from suggested procedure and its comparison with those of other spectrophotometric methods used for the determination of intended compounds.

Compound	Method	Linearity range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Ref.
QN	Derivative Spectrophotometry	1.50 – 17.90	0.500	1.670	This work
	UV-Spectroscopy	16.0 - 100	---	---	[31]
	UV-Spectroscopy	5.0 – 30.0	0.660	2.000	[32]
	Fluorimetry	1.0 – 10.0	0.990	1.460	[33]
	Fluorimetry	5.0 – 40.0	3.600	10.800	[34]
AN	Derivative Spectrophotometry	5.40 – 27.00	1.490	4.970	This work
	Visible Spectrophotometry	1.00 – 10.00	-	-	[35]
MCP	Derivative Spectrophotometry	1.30 – 21.50	0.670	2.240	This work
	UV-Spectroscopy	5.00 – 30.00	0.900	2.800	[18]
	Visible Spectrophotometry	1.35 – 40.37	0.669	2.230	[36]
	Visible Spectrophotometry	0.336 – 3.363	0.046	0.155	[37]
	Visible Spectrophotometry	4.00 – 56.00	0.066	0.219	[38]

Analysis of binary mixtures and spiked urine samples

QN, AN and MCP were simultaneously determined in binary synthetic mixtures and spiked urine samples in order to guarantee the suggested procedure's selectivity and accuracy. Multiple measurements of each concentration were used to verify of the repeatability of proposed analytical process's (n = 10). Relative standard deviation (%RSD) and other statistical parameters of the proposed method were calculated and tabulated in Table 3. Accuracy is the measure of exactness of the analytical method, and it has been proven for the proposed method through preparing solutions at three different concentrations of QN, AN and MCP. The accuracy of the proposed method was determined during recovery experiments. The recovery studies were carried out ten times by the standard addition method, and the percentage recoveries with standard deviation were calculated.

Table 3. Simultaneous quantification of QN, AN and MCP in synthetic binary QN–AN and QN–MCP mixtures.

QN-AN mixture										QN-MCP mixture									
Taken µg		Found* µg		RSD		Recovery (%)		Average Recovery (%)		Taken µg		Found* µg		RSD		Recovery (%)		Average Recovery (%)	
QN	AN	QN	AN	QN	AN	QN	AN	QN	AN	QN	MCP	QN	MCP	QN	MCP	QN	MCP	QN	MCP
20	4	20.18	4.03	0.07	0.03	100.90	100.75			30	1	30.52	1.02	0.05	0.01	101.73	102.00		
18	6	18.15	5.92	0.05	0.03	100.83	98.67			26	2	26.03	1.95	0.05	0.02	100.12	97.50		
16	8	15.16	8.10	0.01	0.01	94.75	101.25			24	4	24.17	4.05	0.09	0.03	100.71	101.25		
12	10	12.03	10.03	0.01	0.02	100.25	100.30			20	6	20.02	6.09	0.08	0.01	100.10	101.50		
10	12	9.91	12.10	0.02	0.01	99.10	100.83	99.47	100.32	16	8	15.86	7.83	0.03	0.04	99.13	97.88	99.87	100.32
8	14	7.97	13.86	0.02	0.04	99.63	99.00			12	10	11.99	10.06	0.01	0.02	99.92	100.60		
6	16	5.88	16.07	0.02	0.05	98.00	100.44			8	12	8.07	12.17	0.03	0.03	100.88	101.42		
4	18	4.01	18.11	0.01	0.04	100.25	100.61			4	14	4.01	13.92	0.01	0.06	100.25	99.43		
2	20	2.03	20.21	0.02	0.07	101.50	101.05			1	16	0.96	16.21	0.02	0.05	96.00	101.31		

*Average of 10 determinations.

CONCLUSION

In the present article, a binary mixture of QN-AN or QN-MCP was prepared and completed to the required volume using 0.1 M HCl. Using the "zero-crossing" measurement technique, first-derivative spectrophotometric process was used to determine QN, AN and MCP in prepared binary mixtures: QN-AN and QN-MCP. The obtained results were very useful for the determination of QN (1.50-17.90 µg/mL) in the presence of 4.32 µg/mL of AN and AN (5.40-27.00 µg/mL) in the presence of 8.96 µg/mL of QN using the first-derivative spectra of QN and AN binary mixture. The first-derivative spectra of QN and MCP allowed the determination of QN (12.00–95.37 µg/mL) in the presence of 5.40 µg/mL of MCP, and MCP (1.30-21.50 µg/mL) in the existence of 29.88 µg/mL of QN in the binary mixture of QN and MCP. The statistical processing of experimental data was used to perform a rigorous evaluation of the recommended approaches.

Conflict of interest

The authors declare no conflict of interest.

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