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Derivative spectrophotometric analysis of benzophenone (as an impurity) in phenytoin

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Abstract

Three simple and rapid spectrophotometric methods were developed for detection and trace determination of benzophenone (the main impurity) in phenytoin bulk powder and pharmaceutical formulations. The first method, zero-crossing first derivative spectrophotometry, depends on measuring the first derivative trough values at 257.6 nm for benzophenone. The second method, zero-crossing third derivative spectrophotometry, depends on measuring the third derivative peak values at 263.2 nm. The third method, ratio first derivative spectrophotometry, depends on measuring the peak amplitudes of the first derivative of the ratio spectra (the spectra of benzophenone divided by the spectrum of 5.0 μg/mL phenytoin solution) at 272 nm. The calibration graphs were linear over the range of 1-10 μg/mL. The detection limits of the first and the third derivative methods were found to be 0.04 μg/mL and 0.11 μg/mL and the quantitation limits were 0.13 μg/mL and 0.34 μg/mL, respectively, while for the ratio derivative method, the detection limit was 0.06 μg/mL and the quantitation limit was 0.18 μg/mL. The proposed methods were applied successfully to the assay of the studied drug in phenytoin bulk powder and certain pharmaceutical preparations. The results were statistically compared to those obtained using a polarographic method and were found to be in good agreement.

Background

Phenytoin is a hydantoin antiepileptic used to control partial and generalized tonic-clonic seizures. It has also been used in treatment of trigeminal neuralgia and cardiac arrhythmias [1]. Benzophenone can be used as fixative for perfumes [2] and soaps, it prevents the ultraviolet (UV) light from damaging scents and colors in products, It can also be added to the plastic packaging as a UV blocker [3]. Benzophenone used in the manufacture of antihistamines, hypnotics and insecticides [2] and so it considered as a potential impurity in diphenhydramine hydrochloride [4,5], phenytoin bulk powder [4,5] and phenytoin dosage forms [4,5].

Benzophenone is irritating to the eyes, the skin and the respiratory tract [6,7] and at excessive exposure, CNS disturbance and coma may be noted [6]. Chhabra [8] and Rhodes *et al*. [9] found that, the liver is a primary target organ of benzophenone toxicity in rats and mice. The toxicological profile of benzophenone is similar to a number of known hepatocarcinogens, suggesting that benzophenone is a potential liver carcinogen. The kidney was also identified as a target organ of benzophenone toxicity. Estrogenic and antiandrogenic activities of benzophenone were comparatively examined with hormone-responsive reporter assay in various cell lines [10-12].

The United States Pharmacopoeia USP [5] recommended HPLC procedures for the determination of benzophenone as a main impurity in phenytoin. The BP [4] specifies a TLC method to limit benzophenone in phenytoin.

A review of the literature revealed that only one (polarographic) method has been published for the determination of benzophenone, as an impurity, in phenytoin [13]. Although those methods offer a high degree of specificity, the instrumentation limitations preclude their use in routine analysis. To the best of our knowledge, no spectrophotometric methods have been yet described for the determination of benzophenone, as an impurity, in phenytoin. Therefore, it was desirable to develop a simple and fast procedure that could be applied in quality control laboratories for the determination of benzophenone, as an impurity, in phenytoin.

Derivative spectrophotometry offers greater selectivity than does normal spectrophotometry in the simultaneous

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determination of two or more compounds without previous chemical separation [14-16]. The principles and advantages of this technique have been described by O'Haver and Green [17].

Ratio derivative spectrophotometry is based on the use of the first derivative of the ratio spectra. This method was developed by Salinas et al [18]. Berzas Nevado et al. extended this method to resolving ternary mixtures [19].

The present paper describes simple and rapid methods for the determination of benzophenone, as impurity, in phenytoin bulk powder and certain pharmaceutical preparations by zero-crossing first derivative spectrophotometry, zero-crossing third derivative spectrophotometry and ratio first derivative spectrophotometry without prior separation.

Experimental

Apparatus

Spectrophotometric analysis was carried out on a Shimadzu (Kyoto, Japan) UV-1601 PC, UV-Visible double-beam spectrophotometer with matched 1-cm pathlength quartz cells. Suitable settings were: Slit width, 1 nm; scan speed, fast; sampling interval, auto.

The first derivative spectra of benzophenone were derived in the wavelength range (250-350) nm using $\Delta\lambda$ = 2 nm and scaling factor = 100.

The third derivative spectra of the studied drug were derived in the wavelength range (240-300) nm using $\Delta\lambda$ = 8 nm and scaling factor = 2000.

The ratio derivative spectra were derived in the wavelength range (200-298) nm using $\Delta\lambda$ = 8 nm and scaling factor = 1 for smoothing of ratio spectra and $\Delta\lambda$ = 4 nm for the first derivative of ratio spectra with scaling factor = 10.

Materials and reagents

All the chemicals used were of Analytical Reagent grade and the solvents were of spectroscopic grade.

- Benzophenone was kindly provided by (El-Nasr Pharmaceutical Chemicals Company (ADWIC), Egypt). The purity of the sample was found to be 100.15 ± 0.36 which was determined by applying the reported polarographic[13] method.
- Phenytoin (Aldrich Chemical Co., Ltd., Dorset, England).
- Phenytin® ampoules (labeled to contain 100 mg phenytoin sodium/2 mL ampoule, batch # 95040) were manufactured in The Nile Co. for Pharmaceuticals & Chemical Industries, Cairo, Egypt.
- Phenytin® capsules (labeled to contain 100 mg phenytoin sodium/capsule, batch # 36050) were manufactured in The Nile Co. for Pharmaceuticals & Chemical Industries, Cairo, Egypt.
- Ipanten® capsules (labeled to contain 100 mg phenytoin sodium/capsule, batch # AC1009) were manufactured in ACDIMA International trading, Egypt.
- All Pharmaceutical preparations were purchased from commercial sources.
- Methanol (Prolabo, France).

Standard solutions

Stock solutions (1.0 mg/mL) of each of benzophenone and phenytoin were prepared by dissolving 10.0 mg of each in methanol and completed to 10 mL with the same solvent. Working standard solutions (100.0 μg/mL) were prepared by subsequent dilution to 25 mL with methanol. An additional working solution (10.0 mg/mL) of phenytoin was prepared by the same manner, to be used in the preparation of synthetic mixtures. The solutions were stable for at least 7 days when kept in the refrigerator at 4°c.

Construction of the Calibration Graphs

Daily working standard solutions were prepared from the previously prepared working standard solution by serial dilutions with methanol to contain 1 - 10 μg/mL for benzophenone and to contain 200 μg/mL for phenytoin (final concentrations). The zero order absorption spectra were recorded against a reagent blank (methanol).

The absolute values of the first order derivatives were obtained by zero-crossing technique with measurements at 257.6 nm for benzophenone.

For the determination by third derivative spectrophotometry, the absolute values of the third derivative were measured at 263.2 nm.

For the determination by ratio derivative spectrophotometry, the first derivative of the ratio spectra (the spectra of benzophenone divided by the spectrum of 5.0 μg/mL phenytoin solution) was recorded. The amplitudes at 272 nm were measured.

The derivative amplitudes were then plotted against the final concentrations to get the calibration graphs. Alternatively, the corresponding regression equations were derived.

Application of the Proposed Methods to the Analysis of Benzophenone in Prepared Synthetic Mixtures

Accurately measured aliquots of the suitable working standard solutions of both drugs were transferred into a series of 10-mL volumetric flasks to prepare different synthetic mixtures of benzophenone and phenytoin in the ratios of 0.5% (the BP [4] limits of benzophenone in phenytoin) and 0.1% (the USP [5] limits of benzophenone in phenytoin). The solutions were then diluted with methanol to volume, mixed and aliquots containing suitable concentrations were taken and analyzed as described under *construction of the calibration graphs*.

The concentration of benzophenone was determined using, either the calibration curve or the corresponding regression equation.

Application of the Proposed Methods to the Analysis of Added Benzophenone in Phenytoin Dosage Forms

Capsules

An accurately weighed quantity of the mixed contents of 10 capsules equivalent to 0.5 g of phenytoin was transferred into a small conical flask, and then extracted by sonication for 15 minutes with 15 mL of methanol and filtering into 50-mL volumetric flask. Repeat the extraction with further two 15-mL portions of methanol. The conical flask was washed with several milliliters of methanol. The washings were passed into the same volumetric flask and completed to the volume with the same solvent. The above procedure under *application of the proposed methods to the analysis of benzophenone in prepared synthetic mixtures* was then followed. The nominal content was calculated either from the previously plotted calibration graph or using the corresponding regression equation.

Ampoules

An accurately measured volume of mixed content of 10 ampoules equivalent to 1.0 g of phenytoin base transferred into 100-mL volumetric flask, and then diluted to the mark with methanol. The above procedure under *application of the proposed methods to the analysis of benzophenone in prepared synthetic mixtures* was then followed. The nominal content was calculated either from the previously plotted calibration graph or using the corresponding regression equation.

Results and discussion

The BP [4] limits are 0.5% for benzophenone in bulk powder and in, capsules and ampoules of phenytoin, whereas, the USP [5] limits are 0.1% for the studied drug in phenytoin bulk powder. Moreover the specific absorbance of phenytoin (the major component) is higher than the specific absorbance of benzophenone (the minor component). So, the analysis of such mixture by conventional spectrophotometry was challenging. Therefore, we resorted to first derivative, third derivative and ratio first derivative spectrophotometry in an attempt to detect and determine benzophenone (the main impurity) in phenytoin.

Dilution with different solvents such as 0.1 M HCl, 0.1 M NaOH, methanol and acetonitrile were tried for performance investigations. It was found that; methanol was the best solvent for dilution as it gave the highest sensitivity with good shaped spectrum.

Figure 1(a, b) shows the absorption spectra of benzophenone and phenytoin in methanol which overlap sufficiently to demonstrate the resolving power of the proposed methods.

'Zero-crossing' first derivative spectrophotometry

Because the derivative spectrophotometric technique enhances the detectability of the minor features of the UV absorption spectrum, the first derivative spectra of both benzophenone and phenytoin (Figure 2) displays features which may permit more specific and selective determination of the benzophenone in the presence of phenytoin. The zero-crossing method is the most common procedure for conducting analytical calibration in derivative spectrophotometry, so benzophenone was determined by measurement of its first derivative amplitude at the zero-crossing of phenytoin at 257.6 nm ($^{1}D_{257.6}$; $^{\text{order derivative}}\text{Derivative}_{\text{wavelength measure}}$).

Figure 2 shows the first derivative spectra of benzophenone in concentration range of 1-10 μg/mL with constant phenytoin concentration (200 μg/mL).

'Zero-crossing' third derivative spectrophotometry

Second and fourth derivative spectra were scanned but a reproducible zero crossing point could not be found. Figure 3 shows the third derivative spectra of benzophenone which could be determined by measurement of its third derivative amplitude at the zero-crossing of phenytoin at 259 nm ($^{3}D_{259}$), or at 263.2 nm ($^{3}D_{263.2}$) both with the same sensitivity.

Ratio first derivative spectrophotometry

Figure 4 shows the ratio spectra of different concentrations of benzophenone (spectra divided by the spectrum of a 5 μg/mL of phenytoin) while, Figure 5 shows their first derivatives. As it can be seen, the amplitude at 272 nm ($^{1}DD_{272}$; $^{\text{order derivative}}\text{Derivative Divided}_{\text{wavelength measure}}$) in the ratio derivative spectra corresponds to benzophenone present in the solution, so it can be used for its quantitative determination. The influence of $\Delta\lambda$ for obtaining the first derivative of the ratio spectra was tested to obtain the optimum wavelength interval; $\Delta\lambda$ = 4 nm was considered as suitable for this. For selecting the standard solution as divisor, different concentrations were tested and different calibration curves were obtained.

The best results in terms of signal-to-noise ratio, sensitivity and repeatability were obtained by using the spectrum of 5 μg/mL phenytoin solution as divisor in the determination of benzophenone.

To ensure the validity of the proposed methods, the results were compared with those of a comparison differential pulse (DPP) polarographic method [13]. However USP [5] recommends an HPLC method as limit test for benzophenone in bulk drug only, while BP [4] recommends a thin-layer (TLC) chromatographic method as a limit test for benzophenone in phenytoin bulk powder and dosage forms.

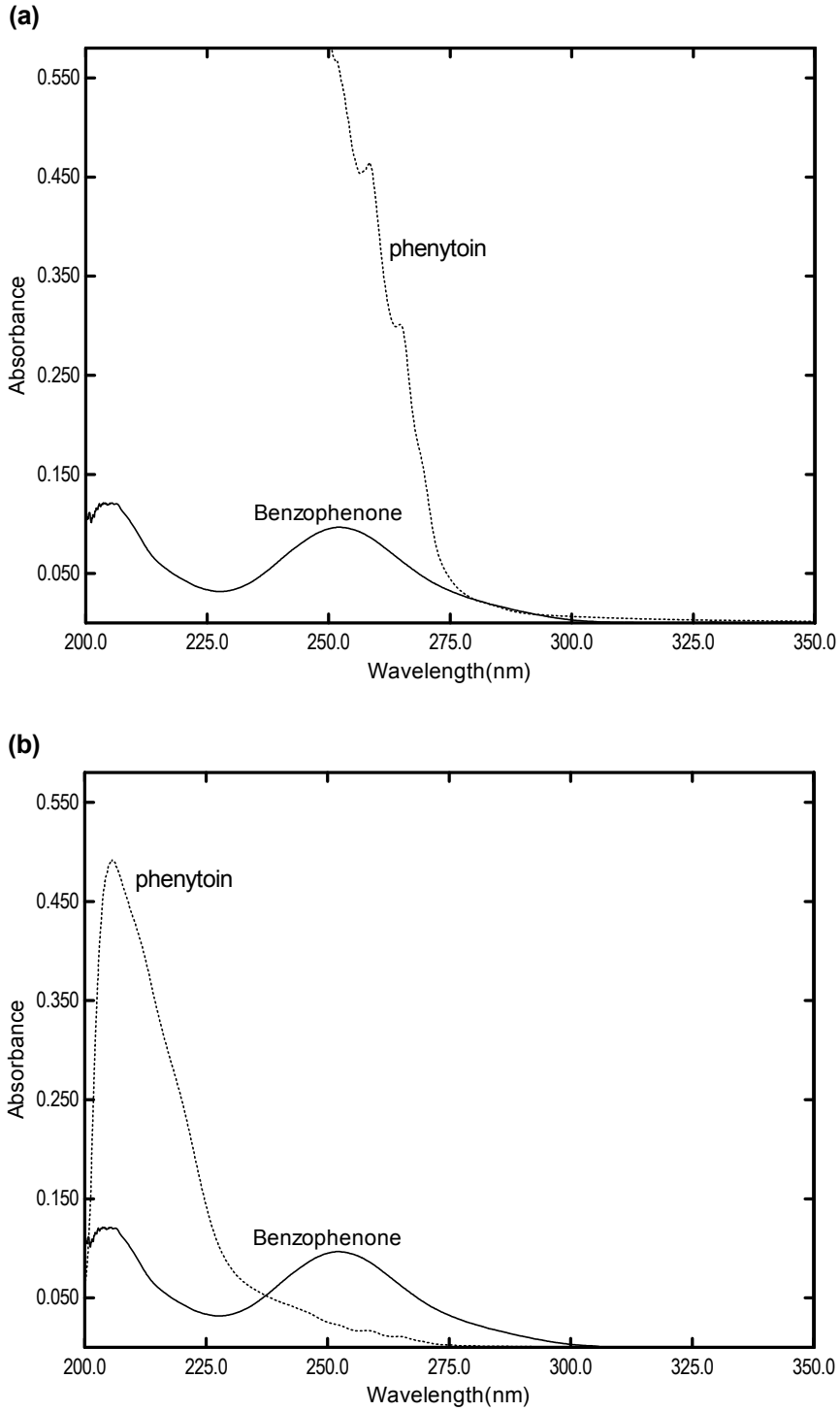

Figure 1 A: Zeroth derivative spectra of 1 μg/mL benzophenone (–) and 200 μg/mL phenytoin (.....) in methanol. B: Zeroth derivative spectra of 1 μg/mL benzophenone (–) and 5 μg/mL phenytoin (.....) in methanol.

The results of the three proposed methods showed no significant differences with those obtained by the comparison polarographic method [13] as regards to accuracy and precision [20].

Validation

Linearity and Range

The calibration graphs for the determination of benzophenone by the proposed methods were constructed by

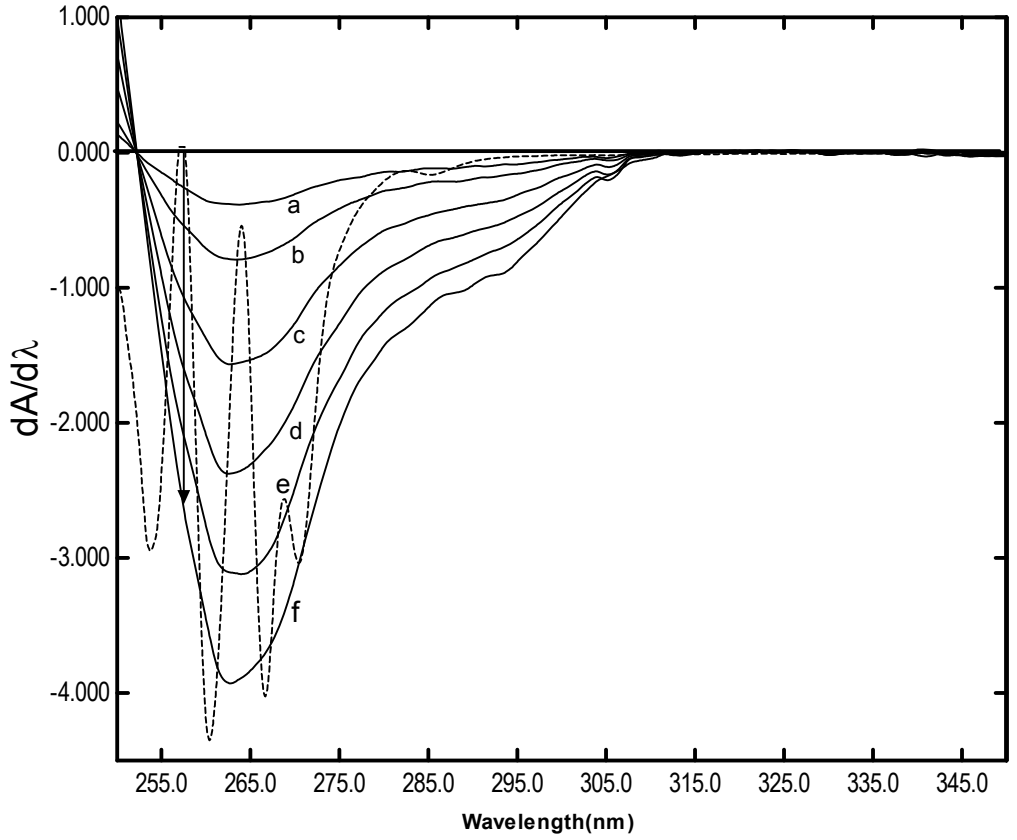

Figure 2 First derivative absorption spectra of benzophenone and 200 μg/mL phenytoin (.....) in methanol.

plotting the derivative amplitudes *versus* the concentrations as shown in Figures 6, 7 and 8. The graphs were found to be rectilinear over the concentration ranges cited in Table 1.

Statistical analysis [20] of the data gave high values of correlation coefficients (*r*) of the regression equations, small values of the standard deviations of residuals ($S_{y/x}$), of intercept (S_a), and of slope (S_b), and small values of

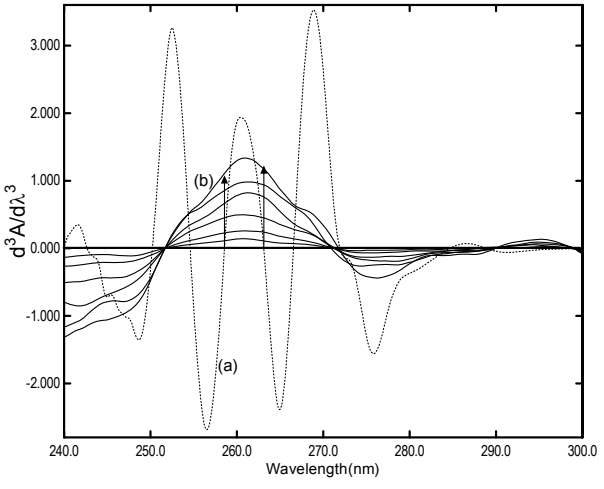

Figure 3 Third derivative spectrum of phenytoin (200 μg/mL) and Series of third derivative spectra with varying concentrations of benzophenone (1- 10 μg/mL).

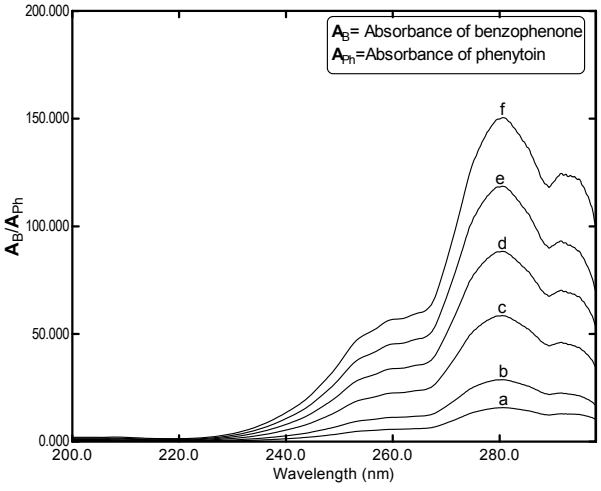

Figure 4 Ratio spectra of benzophenone when 5 μg/mL phenytoin is used as divisor.

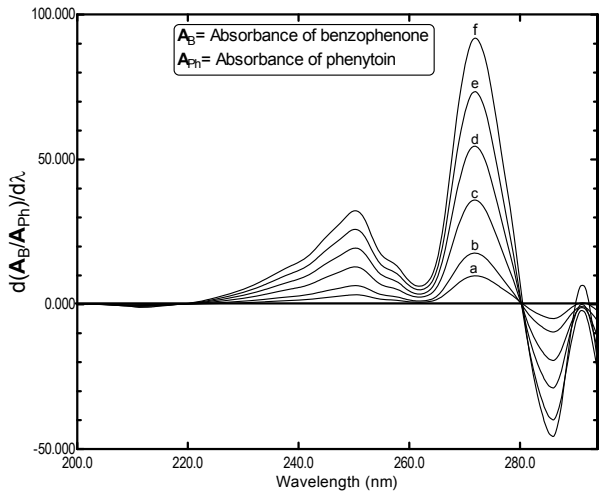

Figure 5 First derivative of the ratio spectra of benzophenone when 5 μg/mL phenytoin is used as divisor.

percentage relative standard deviation and percentage relative error (Table 1). These data proved the linearity of the calibration graphs and the agreement of the results with Beer's law.

Accuracy and Precision

To prove the accuracy of the proposed methods, the results of the assay of benzophenone as impurities in phenytoin bulk powder and its pharmaceutical preparations were compared with those of a reported comparison polarographic method [13]. Moreover, several synthetic mixtures in BP [4] and USP [5] limits ratio were also analyzed. Statistical analysis [20] of the results obtained by the proposed and comparison method using Student's *t*-test (t) and variance ratio *F*-test (F) showed no significant differences between them regarding accuracy and precision, respectively (Tables 2, 3 and 4). Intraday (repeatability) and inter-day (intermediate) precisions were assessed using three concentrations and three replicates of each concentration. The relative

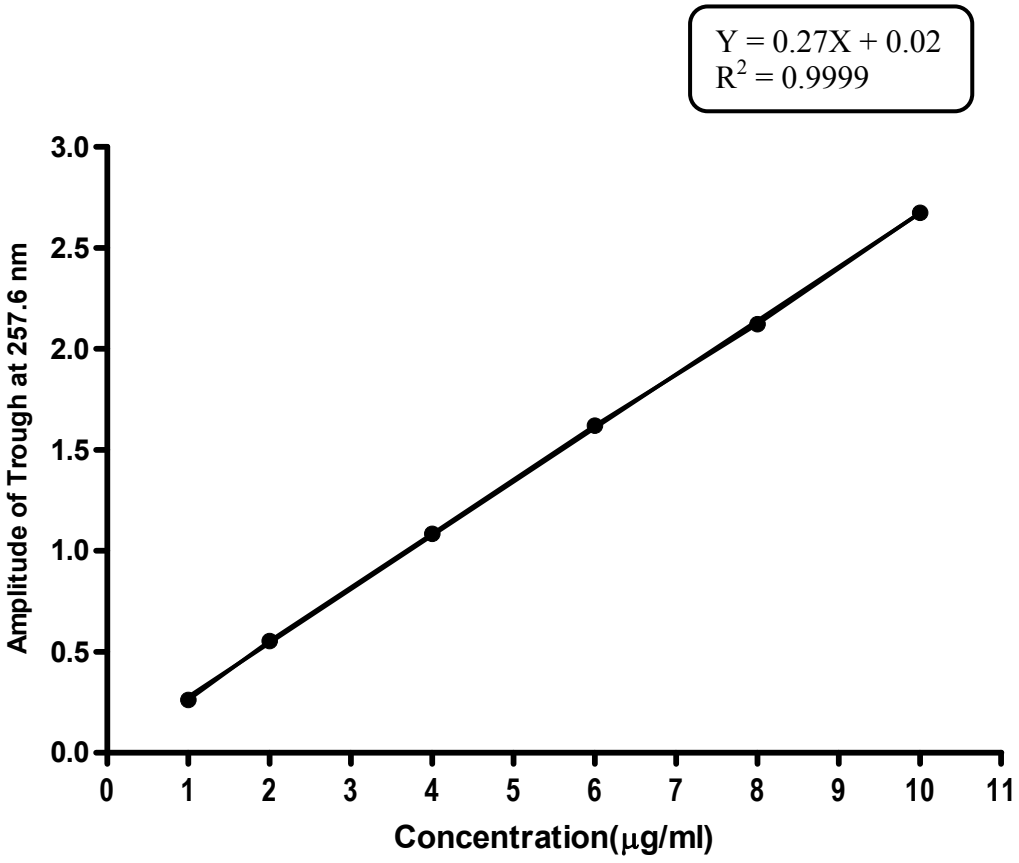

Figure 6 Calibration curves for the determination of benzophenone by zero-crossing first derivative spectrophotometric method at 257.6 nm zero-crossing wavelength.

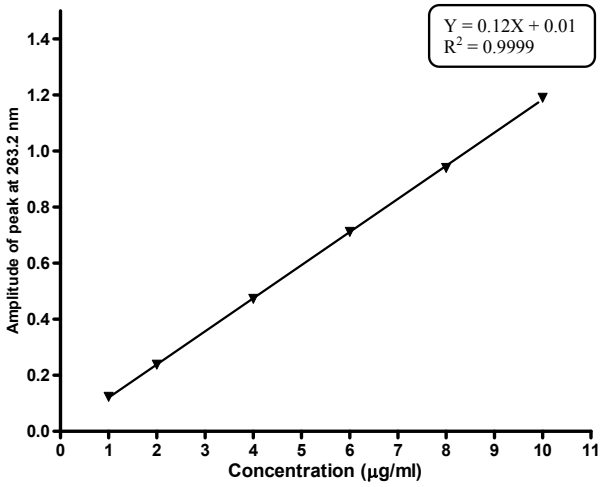

Figure 7 Calibration curve for the determination of benzophenone by zero-crossing third derivative spectrophotometric method at 263.2 nm zero-crossing wavelength.

standard deviations were found to be very small indicating reasonable repeatability and intermediate precision of the proposed methods (Table 5).

Specificity

The specificity of each of the proposed methods was investigated by observing any interference encountered from common dosage form excipients. It was shown that these compounds did not interfere with the results of the proposed methods (Table 4).

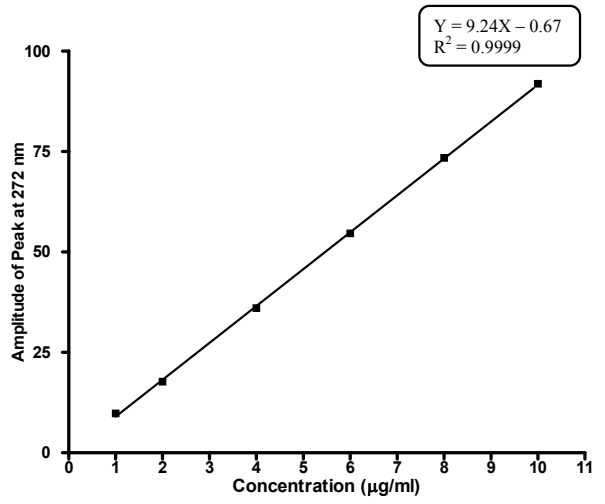

Figure 8 Calibration curve for the determination of benzophenone by the ratio derivative spectrophotometric method at 272 nm.

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

The limit of quantitation (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH Q2)R1) recommendation [21] below which the calibration graph is non linear while the limit of detection (LOD) was determined by evaluating the lowest concentration of the analyte that can be readily detected. The results are shown in Table 1. LOQ and LOD were calculated according to the following equations [21]:

$$LOQ = 10\ S_a/b$$

$$LOD = 3.3\ S_a/b$$

Where S_a is the standard deviation of the intercept of regression line, and b is the slope of the calibration curve.

Ruggedness

To examine the ruggedness of the procedures, the intra-day and inter-day precisions were evaluated as shown in Table 5. The precisions of the proposed methods were fairly high, as indicated by the low values of percentage relative standard deviation (% RSD) for the studied drugs.

Applications

Analysis of Synthetic Mixtures

The proposed methods were successfully applied to the analysis of benzophenone in synthetic mixtures containing the BP [4] and USP [5] limits of benzophenone in phenytoin. The average percent recoveries were based on the average of three replicate determinations. The results obtained were in good agreement with those obtained by the comparison polarographic method [13]. The results are shown in Table 3.

Analysis of Dosage Forms

The proposed methods were successfully applied to the assay of benzophenone (by spiking) in phenytoin ampoules and capsules. The average percent recoveries of different concentrations were based on the average of three replicate determinations. The results obtained showed non-significant differences with those obtained by the comparison polarographic method [13] as shown in Table 4.

Conclusions

The proposed methods could be utilized for the detection and determination of benzophenone, as impurity, in phenytoin bulk powder and its pharmaceutical preparations using simple, reliable, rapid, and economical procedures. The zero-crossing derivative spectrophotometry

Table 1 Analytical performance data of the calibration graphs for the determination of benzophenone by the proposed methods

Parameter	First derivative method (^{1}D)	Third derivative method (^{3}D)	Ratio derivative method (^{1}DD)
Wavelength λ (nm)	257.6	263.2	272
Concentration range (μg/mL)	1-10	1-10	1-10
Intercept (*a*)	20.65×10^{-3}	1.98×10^{-3}	-0.67
Slope (*b*)	0.27	0.12	9.24
Correlation coefficient (*r*)	0.9999	0.9999	0.9999
Standard deviation of the residuals, $S_{y/x}$	4.77×10^{-3}	5.14×10^{-3}	0.21
Standard deviation of the intercept, S_a	3.71×10^{-3}	3.99×10^{-3}	0.16
Standard deviation of the slope, S_b	0.61×10^{-3}	0.66×10^{-3}	0.02
Relative standard deviation, % RSD	0.83	1.10	0.97
Percentage error, % Error	0.34	0.45	0.39
Limit of detection (LOD) (μg/mL)	0.04	0.11	0.06
Limit of quantitation (LOQ) (μg/mL)	0.13	0.34	0.18
D $A^{1\%}$ ($dl.g^{-1}.cm^{-1}$)[a]	2655	1181	9.24×10^4
D ε ($L.mol^{-1}.cm^{-1}$)[b]	4.83×10^4	2.15×10^4	1.68×10^7

[a] Specific absorbance of the derivative mode.

[b] Molar absorptivity of the derivative mode.

Table 2 Assay results for the determination of benzophenone in pure forms

Parameter	Proposed method			Comparison method[13]
	First derivative method (^{1}D)	Third derivative method (^{3}D)	Ratio derivative method (^{1}DD)	
% Found[a]	098.18	102.35	102.03	100.56
	100.41	099.84	100.50	099.89
	100.20	099.63	099.20	100.01
	100.37	100.12	099.66	
	099.81	099.20	100.15	
	099.96	100.51	100.11	
Mean ($\overline{x}$) ± S.D.	099.82 ± 0.83	100.27 ± 1.10	100.27 ± 0.97	100.15 ± 0.36
T	0.64	0.18	0.20	
F	5.48	9.61	7.37	

[a] The average of three determinations.

N.B. Tabulated *t*-value at $P = 0.05$ is 2.36, tabulated *F*-value at $P = 0.05$ is 19.30.

Table 3 Assay results for the determination of benzophenone in synthetic mixtures

Prepared Ratio	Amount taken (μg/mL)		% Found[a]			
	Benzophenone	Phenytoin	Proposed method			Comparison method[13]
			^{1}D	^{3}D	^{1}DD	
0.5%	1.0	200.0	101.63	100.69	099.12	099.60
	2.0	400.0	100.94	102.06	098.69	100.98
	3.0	600.0	099.83	099.94	099.72	098.98
$\overline{x}$ ± S.D.			100.08 ± 0.90	100.90 ± 1.07	099.18 ± 0.52	099.85 ± 1.02
T			1.20	1.22	1.02	
F			1.27	1.10	3.92	
0.1%	1.0	1000.0	099.69	098.56	100.20	099.60
	2.0	2000.0	101.06	099.97	101.09	100.98
	3.0	3000.0	098.91	100.09	099.93	098.98

Table 3 Assay results for the determination of benzophenone in synthetic mixtures *(Continued)*

$\overline{x}$ ± S.D.	099.89 ± 1.08	099.54 ± 0.85	100.41 ± 0.61	099.85 ± 1.02
t	0.04	0.41	0.81	
F	1.13	1.44	2.84	

[a] The average of three determinations.

N.B. Tabulated *t*-value at $P = 0.05$ is 2.78, tabulated *F*-value at $P = 0.05$ is 19.00

Table 4 Assay results for the determination of added benzophenone in phenytoin pharmaceutical preparations

Dosage form	% Found[(a)] of Benzophenone(added)			
	Proposed method			Comparison method[13]
	^{1}D	^{3}D	^{1}DD	
Phenytin® ampoules	099.80	099.25	101.05	099.60
	101.00	099.63	099.25	100.98
	099.95	098.33	100.19	098.98
Mean ($\overline{x}$) ± S.D.	100.25 ± 0.65	099.07 ± 0.67	100.16 ± 0.90	099.85 ± 1.02
T	0.57	1.10	0.39	
F	2.45	2.35	1.29	
Phenytin® capsules	099.00	102.04	100.92	099.60
	099.80	101.69	099.33	100.98
	099.95	100.06	099.92	098.98
Mean ($\overline{x}$) ± S.D.	099.58 ± 0.51	101.26 ± 1.05	100.06 ± 0.80	099.85 ± 1.02
T	0.41	1.66	0.27	
F	4.02	1.07	1.62	
Ipanten® capsules	100.10	101.59	101.15	099.60
	099.10	100.06	100.01	100.98
	099.90	099.65	099.86	098.98
Mean ($\overline{x}$) ± S.D.	099.70 ± 0.53	100.43 ± 1.02	100.34 ± 0.71	099.85 ± 1.02
T	0.23	0.69	0.68	
F	3.74	1.00	2.11	

[a] The average of three separate determinations.

N.B. Tabulated *t*-value at $P = 0.05$ is 2.78, tabulated *F*-value at $P = 0.05$ is 19.00

Table 5 Accuracy and precision data for the determination of benzophenone by the proposed methods

	Parameters	First derivative method (^{1}D)	Third derivative method (^{3}D)	Ratio derivative method (^{1}DD)
Intra-day	% Found[(a)]	099.55	099.68	100.09
		099.96	101.36	101.21
		100.34	099.56	099.23
	Mean ($\overline{x}$)	099.95	100.20	100.18
	S.D.	0.39	1.00	0.99
	%RSD	0.39	1.00	0.99
	%Error	0.16	0.41	0.40
Inter-day	1st day	100.10	102.06	100.31
	2nd day	100.90	101.59	098.99
	3rd day	101.98	100.66	100.11
	Mean ($\overline{x}$)	100.99	101.43	099.80
	S.D.	0.94	0.71	0.71
	%RSD	0.94	0.70	0.71
	%Error	0.38	0.29	0.29

N.B. Each result is the average of three separate determinations.

is more rapid and simple than ratio derivative spectrophotometry; however the ratio derivative spectrophotometry has greater sensitivity and accuracy. The use of standardized spectra as divisors minimizes experimental errors and background noise.

These proposed methods could be regarded as useful alternative to the official USP chromatographic, the BP qualitative TLC and reported polarographic methods in the routine quality control of pharmaceutical formulations, allowing qualitative and quantitative determinations to be simultaneously and rapidly achieved with a relatively inexpensive instrumentation.

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Authors' contributions
MIW designed the proposed method and analyzed the data statistically. MSR proposed, planned and supervised the whole work. ZAS coordinated the study and modified the text. MMS carried out the experimental work. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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