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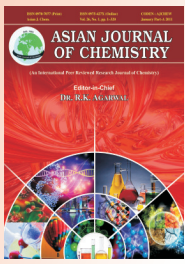

A Validated UV Spectroscopic Method for Simultaneous Determination of Benzil and Benzophenone Impurities in Phenytoin Sodium

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A simple, accurate, precise, economical and reproducible UV spectrophotometric method has been developed for the simultaneous determination of benzophenone and benzil impurities in phenytoin sodium. The method employs formation and solving of simultaneous equation using 222, 257 and 263 nm as the analytical wavelengths. Method was found to be precise and was reproducible as shown from the validation studies.

Keywords: Benzil, Benzophenone, Phenytoin sodium, Simultaneous equation method.

INTRODUCTION

Phenytoin (5,5-diphenylimidazolidine-2,4-dione) 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 is used in the manufacture of hypnotic class of drugs[2] and so it is considered as a potential impurity in phenytoin bulk powder and its dosage forms[3-4]. Benzophenone is irritating to the eyes, the skin and the respiratory tract and at excessive exposure, CNS disturbance and coma may be noted[2]. Chhabra[5] and Rhodes *et al.*[6] have reported that 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.

Phenytoin can also be synthesized by a base catalyzed addition of urea to benzil followed by a benzilic acid rearrangement (1,2 phenyl migration) to form the desired product, known as the Biltz synthesis of phenytoin. Hence benzil is also considered as potential product related impurity in phenytoin bulk powder[7]. Benzil is irritating to eyes and skin on contact. Inhalation causes irritation of the lungs and respiratory system. Inflammation of the eye is characterized by redness, watering and itching. Skin inflammation is characterized by itching, scaling, reddening, or occasionally, blistering[8]. Repeated or prolonged exposure to benzil can produce target organs damage[9].

Benzophenone apart from being a raw material for synthesis of phenytoin sodium, is also reported to be a degradant formed due to oxidative hydrolysis with potassium permanganate[10].

Literature review revealed various HPLC methods[11-13] for the analysis of phenytoin sodium, but methods for analysis of its product related impurities could not be found in the literature. British Pharmacopoeia (BP) specifies a TLC method to limit benzophenone in phenytoin[3]. United States Pharmacopoeia (USP) has recommended HPLC method for the determination of benzophenone as main impurity in phenytoin[4]. A polarographic and a derivative spectrophotometric method have been used for the determination of benzophenone, as an impurity, in phenytoin[14-15]. However, no methods could be found for the simultaneous determination of phenytoin along with two of its potential impurities, benzophenone and benzil. Therefore, it was desirable to develop a simple and sensitive simultaneous UV spectrophotometric method that could be applied in quality control laboratories for the simultaneous determination of benzophenone and benzil as impurities, in phenytoin drug samples. The proposed method is based on the formation and solving of simultaneous equation using 222, 257 and 263 nm as the analytical wavelengths.

EXPERIMENTAL

UV visible double beam spectrometer, Perkin Elmer 25 lambda with spectral bandwidth of 1 nm and a pair of matched quartz cells was used.

All the chemicals used were of analytical reagent grade and the solvents were of spectroscopic grade. Gift sample of phenytoin sodium was kindly provided by Goa Antibiotics Private Limited, Tuem, Pernem Goa. Pure samples of benzophenone and benzil, methanol AR, sodium hydroxide and distilled water was used in the present study.

Solvent system: After assessing the solubility of components in different solvents, a mixture of methanol, 0.1 N sodium hydroxide and distilled water in the ratio of 4:3:3 was used as solvent system for developing spectral characteristics.

Preparation of standard stock solution: Standard stock solution of benzophenone, benzil and phenytoin sodium was prepared by dissolving 10 mg each, in 10 mL of solvent system and final volume adjusted with same solvent. Working standard solutions (100 µg/mL) were prepared by subsequent dilution to 10 mL with same solvent system.

Construction of calibration graphs: Working standard solutions of 10 µg/mL were prepared, heated on water bath for about 1 h, cooled and scanned over the entire UV range 400-200 nm to obtain overlain absorption spectra (Fig. 1). Wavelength of 222, 257 and 263 nm were selected as they are λ_{max} for phenytoin sodium, benzophenone and benzil, respectively (Table-4).

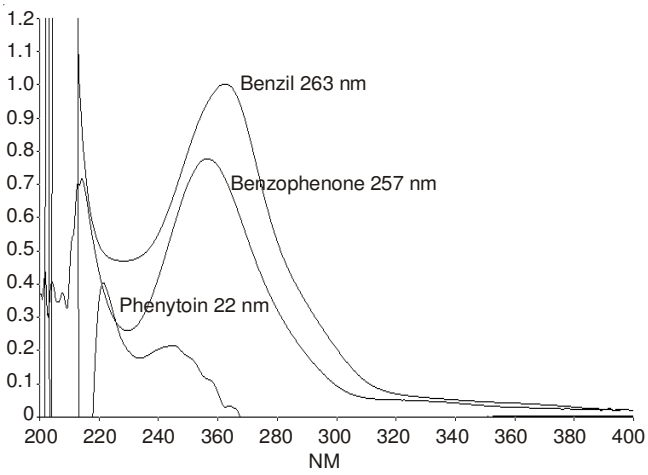

Fig. 1. Overlain spectra of phenytoin sodium, benzil and benzophenone

For linearity study, aliquots of standard solution were further diluted with solvent system to get final working standard of concentration ranging from 5-70, 1-10 and 1-10 µg/mL for phenytoin sodium, benzophenone and benzil, respectively (Table-4). The calibration curves at their respective λ_{max} were prepared from the absorbance values obtained (Figs. 2-4).

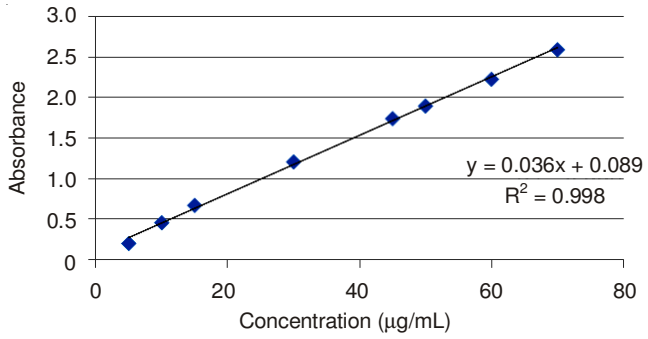

Fig. 2. Calibration curve of phenytoin sodium at 222 nm

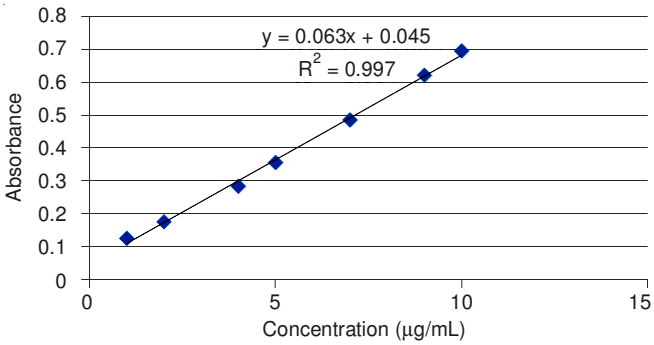

Fig. 3. Calibration curve of benzophenone at 257 nm

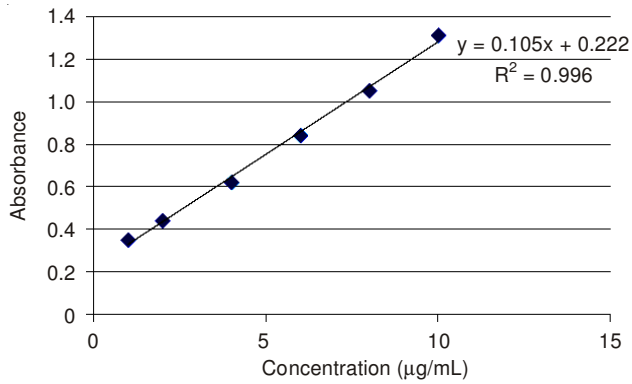

Fig. 4. Calibration curve of benzil at 263 nm

The absorptivity values (A 1 %, 1 cm) were calculated at the selected wavelengths for phenytoin sodium, benzophenone and benzil. The concentration of each component was determined by using simultaneous equation method. The concentration of each component can be calculated using following equation[16].

$$A_1 = a_{X1}\, bc_Y + a_{Y1}\, bc_Y + a_{Z1}\, bc_Z \quad (1)$$

$$A_2 = a_{X2}\, bc_X + a_{Y2} bc_Y + a_{Z2}\, bc_Z \quad (2)$$

$$A_3 = a_{X3} bc_X + a_{Y3}\, bc_Y + a_{Z3} b_{cZ} \quad (3)$$

$$C_X = \frac{A_1(a_{Y2}a_{Z3} - a_{Z2}a_{Y3}) - a_{Y1}(A_2a_{z3} - a_{z2}A_3) + a_{z1}(A_2a_{Y3} - a_{Y2}A_3)}{a_{X1}(a_{Y2}a_{Z3} - a_{Z2}a_{Y3}) - a_{Y1}(a_{X2}a_{z3} - a_{z2}a_{X3}) + a_{z1}(a_{X2}a_{Y3} - a_{Y2}a_{X3})} \quad (4)$$

$$C_Y = \frac{a_{X1}(A_2a_{Z3} - a_{Z2}A_3) - A_1(A_{X2}a_{z3} - a_{z2}a_{X3}) + a_{z1}(a_{X2}A_3 - A_2a_{X3})}{a_{X1}(a_{Y2}a_{Z3} - a_{Z2}a_{Y3}) - a_{Y1}(a_{X2}a_{z3} - a_{z2}a_{X3}) + a_{z1}(a_{X2}a_{Y3} - a_{Y2}a_{X3})} \quad (5)$$

$$C_Z = \frac{a_{X1}(a_{Y2}A_3 - A_2a_{Y3}) - a_{Y1}(a_{X2}A_3 - A_2a_{X3}) + A_1(a_{X2}a_{Y3} - a_{Y2}a_{X3})}{a_{X1}(a_{Y2}a_{Z3} - a_{Z2}a_{Y3}) - a_{Y1}(a_{X2}a_{z3} - a_{z2}a_{X3}) + a_{z1}(a_{X2}a_{Y3} - a_{Y2}a_{X3})} \quad (6)$$

where, A_1, A_2 and A_3 are the absorbance values of the mixture at 222, 257 and 263 nm, respectively. a_{X1}, a_{X2} and a_{X3} are the absorptivities of phenytoin at 222, 257 and 263 nm, respectively. a_{Y1}, a_{Y2} and a_{Y3} are the absorptivities of benzophenone at 222, 257 and 263 nm, respectively. a_{Z1}, a_{Z2} and a_{Z3} are the absorptivities of benzil at 222, 257 and 263 nm, respectively. C_X, C_Y and C_Z are the concentration of phenytoin (x), benzophenone (y) and benzil (z), respectively.

Application of the proposed method for the analysis of benzophenone and benzil in phenytoin drug: Accurately measured aliquots of the suitable working standard solutions of all the three compounds were transferred into a series of 10 mL volumetric flasks to prepare mixtures of benzophenone, benzil and phenytoin sodium. The solutions were then diluted with solvent system to volume. Aliquots of suitable concentrations were prepared and analyzed as described under

construction of the calibration graphs. The concentration of benzophenone, benzil and phenytoin sodium was determined using the simultaneous equation.

The precision of the method (Table-1) was determined by analyzing six replicate mixtures of phenytoin (50 µg/mL) spiked with 1 µg/mL of impurity-1 (benzil) and impurity-2 (benzophenone). The mixture was prepared and analyzed by the proposed method. Results showed insignificant variation demonstrating the method to be repeatable with RSD below 2 %.

TABLE-1
PRECISION OF THE METHOD

S. No.	Concentration (µg/mL)		Recovery (%)	
	Benzophenone	Benzil	Benzophenone	Benzil
1	1.00	1.05	100	105
2	1.00	1.04	100	104
3	1.00	1.00	100	100
4	1.02	1.05	102	105
5	1.05	1.05	105	105
6	1.01	1.04	101	104
Mean	1.013	1.038	101.3	103.8
SD	0.0179	0.0177	0.0179	0.0177
RSD %	1.77	1.64	1.77	1.64

Accuracy for determination of benzil was determined at three levels ranging from 80 to 120 % for the impurities by preparing synthetic mixtures containing benzophenone and benzil in phenytoin sodium, in which the amount of phenytoin sodium (50 µg/mL) and benzophenone (1 µg/mL) was kept constant and the amount of benzil was varied, that is, 0.8, 1 and 1.2 µg for 80, 100 and 120 %, respectively (Table-2). The solutions were prepared in triplicate and the accuracy was described by % recovery which was calculated in the test solution using the simultaneous equation.

Similarly, accuracy for determination of benzophenone was determined at three levels ranging from 80 to 120 % for the impurities by preparing synthetic mixtures containing benzophenone and benzil in phenytoin sodium, in which the amount of phenytoin sodium (50 µg/mL) and benzil (1 µg/mL) was kept constant and the amount of benzophenone was varied, that is 0.8, 1.0, 1.2 µg for 80, 100 and 120 %, respectively (Table-3). The solutions were prepared in triplicate and the accuracy was described by % recovery which was calculated in the test solution using the simultaneous equation.

RESULTS AND DISCUSSION

The proposed method developed for simultaneous analysis of phenytoin sodium, benzil and benzophenone in mixture was found to be simple, accurate, economical and sensitive to be applied in the routine analysis. In the described method there was no additional extraction or separation procedure thereby decreasing the error in quantitation. The method involving formation and solving of simultaneous equation is based on absorptivity coefficient of three components at λ_{max}. Once the equation is framed, absorbance of solution at selected wavelength was measured followed by simple calculation. Framed equation was validated using lab prepared mixed standard of three components which gave satisfactory results.

Benzil is a product related impurity while benzophenone is both product related as well as degradant and when present along with the drug phenytoin sodium, can be simultaneously estimated by the proposed UV spectroscopic method.

TABLE-4
REGRESSION ANALYSIS OF CALIBRATION CURVES AND SUMMARY OF VALIDATION PARAMETERS

Parameters	Phenytoin	Benzophenone	Benzil
Wavelength (nm)	222	257	263
Beer's law limit (µg/mL)	5-70	1-10	1-10
Sandell's sensitivity	0.0247	0.00797	0.00284
Regression equation*	y = 0 .036x + 0.089	y = 0.063x + 0.045	y = 0.105x + 0.222
Intercept (a)	0.089	0.045	0.222
Slope (b)	0.036	0.063	0.105
Correlation coefficient (r)	0.998	0.997	0.996
Relative standard deviation (RSD)	1.07	1.87	0.638

where, *y = a + bx, x is the concentration of the analyte and y is the absorbance value.

Conclusion

The method developed was found to be accurate, precise and simple and could be utilized for determination of benzophenone and benzil as impurity in phenytoin bulk powder.

TABLE-2
ACCURACY STUDY FOR BENZIL IN THE MIXTURE

Sr. no	Amount benzil added to the mixture (µg/mL)	Level of addition (%)	Total amount of benzil (µg/mL)	Concncentration benzil found (µg/mL)	Mean recovery (%) (n = 3)	RSD (%)
1.	0.8	80	1.8	1.79	99.44	0.90
2.	1.0	100	2.0	2.1	105	0.80
3.	1.2	120	2.2	2.2	100	0.94

TABLE-3
ACCURACY STUDY FOR BENZOPHENONE IN THE MIXTURE

Sr. no	Amount benzophenone added to the mixture (µg/mL)	Level of addition (%)	Total amount of Benzophenone (µg/mL)	Concncentration benzophenone found (µg/mL)	Mean Recovery % (n = 3)	RSD (%)
1.	0.8	80	1.8	1.74	96.6	0.92
2.	1.0	100	2.0	2.07	103	0.85
3.	1.2	120	2.2	2.28	103	0.97

The use of simultaneous equation method for analysis of three component mixture allows the determination of benzophenone and benzil as impurity in presence of phenytoin sodium. The proposed method is a useful alternative to the official chromatographic (USP) and qualitative TLC (BP) method in the routine quality control of phenytoin for detection and determination of benzophenone and benzil allowing qualitative and quantitative determination to be simultaneously and rapidly performed with relatively low-cost instrumentation.

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REFERENCES

1. S. Sweetman, Martindale: The Complete Drug Reference, The Pharmaceutical Press, London, p. 495 (2009).
2. http://www.cdc.gov/niosh/ipcsneng/neng0389.html/international/ chemical safety cards for benzophenone.
3. British Pharmacopoeia Commission, The British Pharmacopoeia, The Stationery Office, London, vol. 2, pp. 1614-1615 (2009).
4. United States Pharmacopeial Convention: The United States Pharmacopoeia 32, the National Formulary 27, Rockville, MD, USA, vol. 3, p. 3291 (2009).
5. R.S. Chhabra, *National Toxicology Program*, **61**, 6 (2000).
6. M.C. Rhodes, J.R. Bucher, J.C. Peckham, G.E. Kissling, M.R. Hejtmancik and R.S. Chhabra, *Food Chem. Toxicol*., **45**, 843 (2007).
7. http://en.wikipedia.org/wiki/Benzil.
8. http://www.spectrumchemical.com/MSDS/TCI-B0050.pdf/Benzil Spectrum chemical.
9. http://www.sciencelab.com/msds.phpmsdsId=9923047/MSDS for Benzil.
10. Z. Jung and J. Silhankova, *Farm. Obz*., **47**, 349 (1978).
11. A. Khedr, M. Moustafa, A.B. Abdel-Naim, A. Alahda and H. Mosli, *Anal. Chem. Insights*, **3**, 61 (2008).
12. A. Varaprasad, N. Sriram, A.G.I. Blessing, M. Jawahar and S. Thangamuthu, *Int. J. Biol. Pharm. Res*., **3**, 126 (2012).
13. E. Seyhan, U. Ahmet and O. Tuncel, *Hacettepe Universitesi Eczacilik Fakultesi Dergisi*, **17**, 55 (1997).
14. O.A. Razak, A.A. Gazy and A.M. Wahbi, *J. Pharm. Biomed. Anal*., **28**, 613 (2002).
15. M. Walash, M. Rizk, Z. Sheribah and M. Salim, *Chem. Cent. J*., **5**, 85 (2011).
16. R. Sharma and K. Mehta, *Indian J. Pharm. Sci*., **72**, 527 (2010).