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Polarographic determination of phenytoin and benzophenone (as impurity) in pharmaceutical preparations

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Abstract

A differential pulse polarographic method is described for detection and trace determination of benzophenone (the main impurity) in phenytoin powder. The method depends upon the polarographic activity of benzophenone in Britton–Robinson buffer pH 5.6. The limit of detection was found to be 2.5×10^{-6} μg ml^{-1}. Phenytoin has been analysed polarographically after oxidation with alkaline permanganate to give benzophenone; the limit of detection was found to be 6×10^{-6} μg ml^{-1}.

Keywords: Polarography; Phenytoin; Benzophenone; Purity test

1. Introduction

Phenytoin is a hydantoin antiepileptic used to control partial and generalized tonic seizures [1]. The drug and its pharmaceutical preparations are official in both USP 24 and BP 98. The USP describes HPLC procedures for its assay and for the determination of benzophenone as a main impurity [2]. The BP specifies a TLC method to limit benzophenone in phenytoin and a non-aqueous or gravimetric assay for the determination of phenytoin in pharmaceutical preparations [3]. To our knowledge, no other literature has appeared about the qualitative or quantitative analysis of benzophenone in phenytoin.

Phenytoin has been assayed, in pharmaceutical preparations, spectrophotometrically using first [4], second [5] derivative spectra and computer aided indicator absorbance ratio [6] methods. The drug was also determined by potentiometric [7] and oscillopolarographic [8] titration. A TLC method [9] has also been reported. In biological fluids phenytoin has been determined by HPLC [10–12]. The detection limit of the most recent procedure was 0.05 μg ml^{-1} [12]. The polarographic measurement of phenytoin nitro derivative enabled the determination of the drug in blood with a relatively low sensitivity of 1–2 μg ml^{-1} [13]. Various reported methods for the drug analysis were based on the oxidation of phenytoin to benzophenone. The formed benzophenone has been extracted in hydrocarbon solvents then measured spectrophotometrically [14] or fluorimetrically [15] with detection limits of

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1 and 0.05 μg ml^{-1}, respectively. Korany et al. developed an extraction–spectrophotometric method using dicyclohexano-24-crown-8 to solubilize the potassium permanganate in cyclohexane chloroform mixture. The formed benzophenone has been directly measured with a limit of detection of 12 μg ml^{-1} [16].

In view of the fact that no analytical method has been published for the determination of benzophenone in phenytion, a precise, simple and sensitive differential pulse polarographic assay for the determination of benzophenone in phenytoin has been proposed. Furthermore, an indirect differential pulses polarographic assay of phenytoin in pharmaceutical preparations after oxidation with alkaline permanganate has also been developed.

2. Experimental

2.1. Apparatus

The polarographic measurements were made with a Metrohm 693 VA Processor and a Metrohm 694 VA Stand. The three electrode system was composed of a multimode electrode, which was used in the dropping mercury electrode mode, a Ag/AgCl (3 M KCl) reference electrode and a Pt auxiliary electrode. Polarography was performed in the differential pulse mode. A pulse amplitude of -50 mV was used with a sweep rate of 10 mV s^{-1} and a pulse interval of 0.6 s.

The pH was measured using Gerate pH meter CG 710, calibrated with standard buffers at room temperature.

2.2. Reagents and materials

- Phenytoin and benzophenone were kindly provided by Alexandria Co. for Pharm. and Chem. Industries, Alexandria, Egypt.
- Analytical reagent grade of methanol (BDH) and *n*-heptane (BDH) were used.
- Alkaline potassium permanganate was prepared as 4% w/v in 7 M sodium hydroxide.
- Britton–Robinson buffer pH 5.6 [17].
- Standard stock solutions (0.1 mg ml^{-1}) of benzophenone and phenytoin were prepared in methanol and 0.01 M sodium hydroxide, respectively. The solutions were found to be stable for at least 2 weeks when stored in the refrigerator.
- Pharmaceutical preparations were purchased from the local market.

2.3. Procedures

2.3.1. General procedure and construction of calibration curves

2.3.1.1. For benzophenone. Aliquots ranging from 0.05 to 0.3 ml from the standard stock solution were transferred into a set of 10 ml volumetric flasks. The volume was made up to 2 ml with methanol, completed to the mark with Britton–Robinson buffer pH 5.6 and mixed. The content of each flask was poured into the polarographic cell and purged with nitrogen for 5 min. The differential pulse polarograms were recorded from -800 to -1200 mV, at a sweep rate of 10 mV s^{-1}.

2.3.1.2. For phenytoin. Accurately measured volumes from phenytoin stock standard solution were transferred into separate conical flasks followed by 20 ml of the alkaline permanganate solution. The flasks were heated in a thermostated water bath at 70 °C for 10 min. The solution was cooled and transferred quantitatively into a 100 ml separatory funnel, then extracted three times each with 5 ml *n*-heptane. The extracts were combined and evaporated at room temperature under a stream of nitrogen. The residue was dissolved in 2 ml methanol and the volume was made up to 10 ml with Britton–Robinson buffer pH 5.6. The polarographic measurement was performed as under the general procedure for benzophenone.

2.3.2. For determination of benzophenone in phenytoin powder

Phenytoin (0.4 g) was shaken with 10 ml methanol and warmed in a water bath with shaking. Two milliliters from the supernatent solution

were pipetted into a 10 ml volumetric flask, completed to the mark with Britton–Robinson buffer pH 5.6 and mixed. The polarographic measurement for the resulted turbid solution was performed as under general procedure for benzophenone.

2.3.3. Assay of pharmaceutical preparation

2.3.3.1. Capsules. The content of 20 capsules were weighed and mixed.

For benzophenone. The procedure for the determination of benzophenone in phenytoin powder was followed using a quantity of the content of capsules equivalent to 0.4 g of phenytoin sodium.

For phenytoin. An accurately weighed amount of the content of the capsules equivalent to about 10 mg phenytoin was shaken with 50 ml of 0.01 M sodium hydroxide for 15 min and diluted to 100 ml with the same solvent. Different aliquots from the resulted solution were pipetted and processed as under the general procedure.

2.3.3.2. Oral suspension

For benzophenone. A quantity of the oral suspension containing 30 mg of phenytoin was mixed well with 5 ml of water and 2 ml of 2 M hydrochloric acid and extracted with five 20-ml quantities of ether. The combined ether extracts were washed with three 10-ml quantities of water and evaporated to dryness. The residue was dissolved in 2 ml methanol and completed to 10 ml with Britton–Robinson buffer pH 5.6, then measured as precedent.

For phenytoin. 5-ml of the oral suspension was transferred into a 100-ml volumetric flask and mixed with 60 ml 0.01 M sodium hydroxide. The flask was shaken automatically for 15 min. The volume was completed to the mark with the same solvent. To different aliquots from the resulted suspension the general procedure for phenytoin was applied.

3. Results and discussion

3.1. Determination of benzophenone in phenytoin

Benzophenone in Britton–Robinson buffer (pH 5.6) containing 20% methanol (as solubilizer) produces a well defined cathodic differential pulse peak at −1138 mV (vs. Ag/AgCl) (Fig. 1), due to reduction of its keto group. Phenytoin is polarographically inactive, and it lacks cathodic peak. Therefore, benzophenone could be easily detected and quantitated in phenytoin powder using the sensitive differential pulse polarographic technique.

Reduction of benzophenone at DME is pH dependent; because a proton is involved in the reduction. The peak potential (E_p) is shifted to more negative values upon increasing the pH and vanishes at pH 9.15. Maximum sensitivity was obtained at pH 5.6 (Fig. 2).

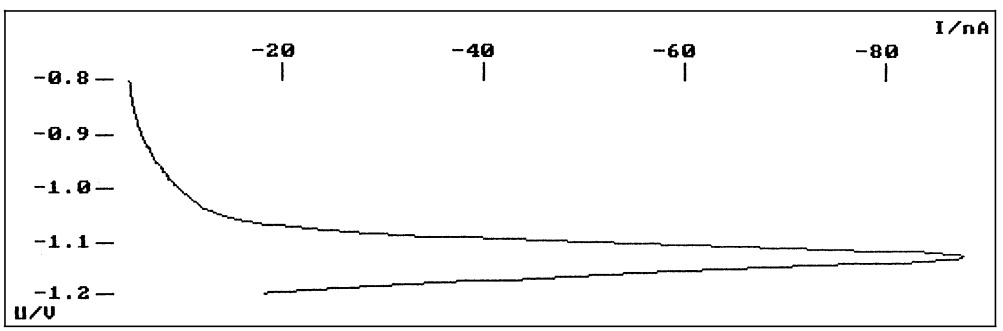

Fig. 1. Differential pulse polarogram of benzophenone (1 μg ml^{-1}) in Britton–Robinson buffer pH 5.6.

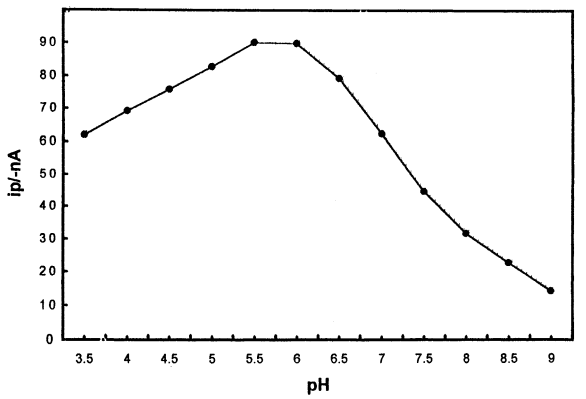

Fig. 2. Influence of pH of Britton–Robinson buffer on the peak current of benzophenone (1 μg ml^{-1}).

3.1.1. Validation of the proposed procedure

- A linear relationship was found between the peak current $(ip)(nA)$ and benzophenone concentration C (μg ml^{-1}) over the concentration range 0.5–3.0 μg ml^{-1}

  $C = 0.526 + 71.012ip.$

  The good linearity is evident from the value of the variance around the slope ($S_b^2 = 1.04$) and correlation coefficient of 0.9996.
- The limit of detection (LOD) has been calculated (3σ; σ is the standard deviation of blank) and validated experimentally. It was found to be 2.5×10^{-6} μg ml^{-1}, indicating high sensitivity of the method.
- The value of the limit of quantitation (LOQ) at a RSD = 2% was fond to be 0.4 μg ml^{-1}.
- The accuracy of the method was assessed by calculating the percentage recoveries of benzophenone spiked in phenytoin above and below the allowable pharmacopoeial limit. The mean percentage recovery was $100.3 \pm 0.60\%$.
- The precision as repeatability was calculated as relative standard deviation of the assay results of three different concentrations each in three replicates and was found to be 0.43%.
- Ruggedness: Varying the pH from 5.4 to 5.8 causes deviation from the optimum value by 0.2–0.9%. Purging of nitrogen for 3, 4, 5 and 6 min has been tested; the polarographic wave has been found to not be affected.
- No interference was found from the commonly used capsule excipients such as lactose, starch, magnesium stearate, microcrystalline cellulose and suspension excipients such as gum, avicel, carboxymethyl cellulose, and sodium lauryl sulfate.

3.1.2. Analytical application

The proposed method has been applied to the determination of benzophenone in capsules and oral suspension of phenytoin. The percentage of benzophenone in phenytoin was found to be 1.7×10^{-3} and 4.5×10^{-3}% w/w, respectively (the BP limits are 0.5 and 0.2%, respectively) (Table 1). In bulk powder of phenytoin, benzophenone was detected. Its concentration was found to be below the LOQ specified above (the BP and USP limits are 0.5 and 0.1%, respectively). The results were in good agreement with the BP(98) TLC method, which showed that the benzophenone concentrations were below the limit.

3.2. For phenytoin

Alkaline permanganate oxidation of phenytoin gives benzophenone [18]. This has been utilized to develop a sensitive polarographic method for de-

Table 1
Determination of benzophenone (as impurity) in pharmaceutical preparation

Commercial products	Benzophenone found (%)[a]
Epanutin capsules[b] (30 mg/Cap.)	1.70×10^{-3}
	1.73×10^{-3}
	1.69×10^{-3}
	1.75×10^{-3}
	1.68×10^{-3}
Mean ± S.D.	$1.7 \times 10^{-3} \pm 2.9 \times 10^{-5}$
Epanutin suspension[b] (30 mg/5 ml)	4.60×10^{-3}
	4.30×10^{-3}
	4.67×10^{-3}
	4.50×10^{-3}
	4.40×10^{-3}
Mean ± S.D.	$4.5 \times 10^{-3} \pm 1.4 \times 10^{-4}$

[a] Calculated as mg benzophenone per 100 mg phenytoin.
[b] Products of Nile Co. for pharmaceuticals (Cairo, Egypt) under licence of Parke–Davis, Detroit.

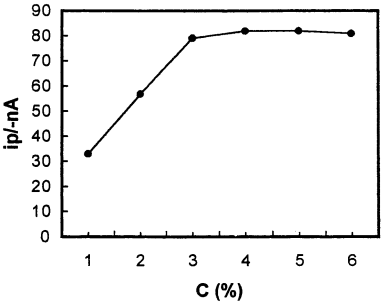

Fig. 3. Influence of $KMnO_4$ concentration (% w/v) on the oxidation reaction (using 2 μg ml^{-1} phenytoin).

termination of phenytoin through functionalization, i.e. the conversion of an electroinactive compound to an active one.

The different experimental parameters affecting the oxidation and the extraction of the formed benzophenone have been carefully studied. It was found that 20 ml of 4% $KMnO_4$ in 7 M sodium hydroxide gave the maximum sensitivity (Figs. 3 and 4). Regarding the heating time and temperature, 10 min at 70 °C gave the best results (Figs. 5 and 6). For the extraction of the resulted benzophenone, different hydrocarbon solvents (cyclohexane, *n*-hexane, *n*-heptane) were examined. All gave the same sensitivity; *n*-heptane was chosen because it was previously used [19].

3.2.1. Validation of the proposed method

Under the above described experimental conditions the peak current is a linear function of phenytoin concentration over the concentration range 1–5 μg ml^{-1}. Regression analysis of the results gave the following equation.

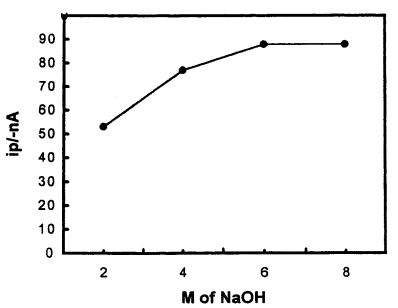

Fig. 4. Influence of NaOH concentration (M) on the oxidation reaction (using 2 μg ml^{-1} phenytoin).

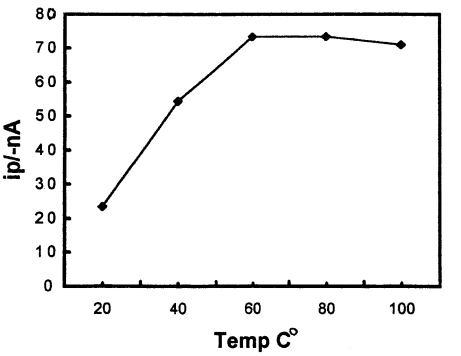

Fig. 5. Influence of heating temperature on the oxidation reaction (using 2 μg ml^{-1} phenytoin).

$$C\ (\mu g\ ml^{-1}) = -2.986 + 47.138ip\ \ (r = 0.999)$$

The variance around the slope (S_b^2) was found to be 1.33.

The accuracy of the method was checked by calculating the relative recoveries of standard phenytoin. The mean percentage recovery was found to be $99.8 \pm 0.7\%$. In order to study the repeatability of the method, standard solutions containing three different concentrations of phenytoin were prepared and three polarographic measurements were made on each reaction product formed according to the recommended procedure. The mean relative standard deviation was found to be 0.41%.

- Ruggedness: varying the concentration of potassium permanganate from 3.5 to 4.5% w/v

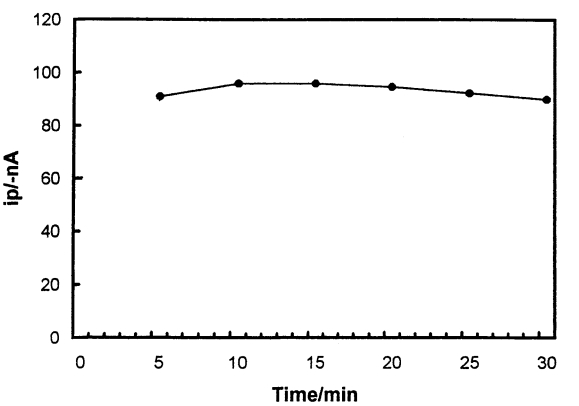

Fig. 6. Influence of heating time on the oxidation reaction (using 2 μg ml^{-1} phenytoin).

in 6–8 M solution of sodium hydroxide, varies the results from 1.7 to 2.9% and 0.1 to 0.6%, respectively. Varying the temperature between 60 and 80 °C for time of 7–15 min varies the results from 1.4 to 2.7% and 0.4 to 1.7%, respectively.

- Interferences: The influence of commonly used capsule excipients (lactose, starch, magnesium stearate, microcrystalline cellulose) and suspension excipients (gum, avicel, carboxymethyl cellulose, sodium lauryl sulfate) was investigated before the determination of the drug in dosage forms. No interference was observed with the proposed method.

3.2.2. Application to pharmaceutical preparations

The proposed method has been successfully applied for the analysis of phenytoin in its commercial tablets and oral suspension. The mean percentages found were 96.9 ± 0.26 and 97.9 ± 0.29%, respectively (Table 2). The results were compared statistically with the published spectrophotometric method [4]. The t- and F-values did not exceed the theoretical ones (Table 2), indicating no significant difference.

Table 2
Determination of phenytoin in pharmaceutical preparation using the proposed polarographic method

Commercial products	Phenytoin found (%)	Reference method
Epanutin capsules[a] (30 mg/Cap.)	96.91	97.00
	96.81	97.30
	97.30	97.60
	96.60	97.30
	97.00	97.10
Mean* ± S.D.	96.92 ± 0.26	97.26 ± 0.23
	$F = 1.28$	
	$t = 2.19$	
Epanutin suspension[a] (30 mg/5 ml)	97.98	98.15
	98.10	98.20
	97.57	97.80
	98.20	98.10
	97.60	97.60
Mean* ± S.D.	97.89 ± 0.29	97.97 ± 0.26
	$F = 1.24$	
	$t = 0.46$	

[a] Products of Nile Co. for pharmaceuticals (Cairo, Egypt) under licence of Parke–Davis, Detroit.

* The average of five determination ± standard deviation. Theoretical value: $t = 2.31$ at the 95% confidence level. Theoretical value: $F = 6.4$ at the 95% confidence level.

4. Conclusion

The proposed polarographic method for the determination of benzophenone, as impurity, in phenytoin and its products is of the same specificity when compared with the HPLC method of the USP 24. The peak potential is characteristic for benzophenone. The proposed method is also as sensitive as the HPLC method of the USP. The BP method is only a qualitative one. The proposed polarographic procedure for the indirect determination of phenytoin is 10^4 times more sensitive than the published HPLC and fluorimetric methods (the lowest LOD of the reported methods is 0.05 μg ml^{-1} [12,15]; the LOD of the proposed method is 6×10^{-6} μg ml^{-1}). The present method is of higher sensitivity compared with the reported indirect polarographic procedure [13] which depends upon nitration prior to analysis. Being an indirect method, this constituted a disadvantage for the analysis of phenytoin. The chromatographic method may be more selective; yet the practical advantages of the polarographic technique (rapidity, simplicity and cheapness) make it very suitable for the routine determination of benzophenone in phenytoin and phenytion in its dosage form.

References

[1] K. Parfitt (Ed.), Martindale, the Complete Drug Reference, 32d ed., Pharmaceutical Press, 1993, p. 352.
[2] United States Pharmacopeia 24, United States Pharmacopeial Convention, Asian Edition, the Board of Trustees, 2000, pp. 1322–1326.
[3] British Pharmacopoeia, The Stationary Office, London, 1998, pp. 1033–1035, 1871, 1872.
[4] A.A.M. Wahbi, A.M. Al Julani, Anal. Lett. 18 (B11) (1985) 1375–1380.
[5] P. Mura, G. Santoni, S. Pinzauti, Pharm. Acta Helv. 62 (8) (1987) 226–228.

[6] S. Lui, R. Li, P. Guo, Y. Yang, Yaowu. Fenxi. Zazhi. 10 (2) (1990) 100–102.
[7] S.F. Chen, C.H. Yang, H.B. Duan, Yaoure Fenxi Zazhi 14 (2) (1994) 60–73.
[8] Z. Zhang, Y. Ding, G. Shen, H. Lin, R. Yu, Yaoure Fenxi Zazhi 9 (2) (1989) 99–100.
[9] H.Y. Abou-Enein, V. Serignese, Anal. Lett. 27 (4) (1994) 723–729.
[10] M.T. Maya, A.R. Farinha, A.M. Lucas, J.A. Morais, J. Pharm. Biomed. Anal. 10 (10–12) (1992) 1001–1006.
[11] N. Bahal, M.C. Nahata, J. Liq. Chromatogr. 16 (5) (1993) 1135–1142.
[12] F.H. Qiu, L. Liu, Y. Luo, Y.Q. Lu, Sepu. 13 (3) (1995) 218–219.
[13] M.A. Brooks, J.A.F. De Silva, M.R. Hackman, Anal. Chim. Acta 64 (1973) 165–175.
[14] W.A. Dill, L. Chucot, T. Chang, A.J. Glazko, Clin. Chem. 17 (1971) 1200.
[15] Lce.D. Morrison, C.M. O'Donnell, Anal. Chem. 46 (8) (1974) 1119–1120.
[16] M.A. Korany, M.A. Bedair, R.S. Haggag, Talanta 46 (1998) 9.
[17] C. Lentner, Geigy Scientific Tables, 8th ed., Ciba-Geigy, 3 (1984) 58–60.
[18] J. Philip, J. Halcomb, S.A. Fusari, Phenytoin, in: K. Florey (Ed.), Analytical Profiles of Drug Substances, Academic Press, New York, 1984, p. 429.
[19] J. Wallace, Anal. Chem. 40 (1968) 978.