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Spectrophotometric Estimation of Phenylephrine Hydrochloride via Oxidative Coupling Reaction with p-Aminobenzophenone

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ABSTRACT

In this research, a rapid, simple and accurate spectrophotometric approach was described for the estimation of phenylephrine hydrochloride in the pure and in its drug forms. The suggested method was based on the oxidative coupling reaction of phenylephrine hydrochloride with p-aminobenzophenone using potassium periodate as an oxidant. A taupe-red dye was formed at room temperature and showed maximum absorption at 512 nm. The linearity of the standard calibration curve was compatible with Beer's law within the concentration range of 2.0-20 µg/mL with a determination coefficient (r^2=0.9986). The apparent molar absorptivity and the sensitivity of Sandell's index were calculated and found to be in the values of $0.552x10^4$ L/mol.cm. and 0.0368 µg/cm^2, respectively. The nature of the resulting dye has been studied between phenylephrine hydrochloride to p-aminobenzophenoneand and it was equal to 1:1. The limits of detection (LOD) and quantification (LOQ) were estimated and found to be 0.0094 and 0.0313 µg/mL, respectively. A relative standard deviation and a relative error were also calculated and they will be in the range of 0.0715 to 0.0216 and -0.0479% to -0.0145%, respectively. The recommended procedure was applied to assay phenylephrine hydrochloride in drops and injection and no interferences were observed from the common additives found in the drugs.

1. Introduction

Phenylephrine hydrochloride (PPH) is in a class of medications called nasal decongestants. It is chemically known as (R)-1-(3-hydroxyphenyl)-2-methylaminoethanol hydrochloride (Figure 1). PPH is a white crystalline powder used in the medical field to relieve sinus congestion and pressure. It can relieve symptoms but cannot release the cause of the symptoms or speed recovery. PPH is also used in the treatment of pharyngitis nasal, allergic conjunctivitis, and nonspecific conjunctivitis (British Pharmacopaeia, 2022; Goodman, 1996).

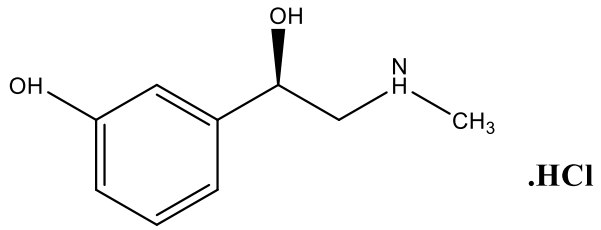

Fig. 1. Phenylephrine hydrochloride (M.Wt.= 203.66 g/mol)

Several analytical techniques have been reported in the literature for the analysis of PPH, such as voltammetry (Yagmur, et al., 2018), RP-HPLC (Dinc Zor, et al., 2017), chemometric-assisted spectrophotometry and RP-HPLC (Al-Shaalan, 2010), high-performance thin layer chromatography (Ragab, et al., 2019), by using carbon nanotubes ceramic electrode (Habibi & Jahanbakhshi,

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2015), electrochemically by using graphene-TiO_2 modified glassy carbon electrode (Li, et al., 2013) and dual wavelength method for the simultaneous determination of PPH in bulk and pharmaceutical dosage forms (Patel, 2013).

Many spectrophotometric methods have also been employed for determining PPH in biological fluids and pharmaceutical forms. Some of these methods were based on the oxidative coupling reactions of PPH with N, N-dimethyl-p-phenylenediamine and sodium persulphate (Radia, et al., 2022), N,N-dimethyl-p-phenylenediamine with $FeCl_3$ in alkaline media (Ahmed, et al., 2020) and 4-aminoantipyrine in the presence of potassium ferricyanide (Aljeboree & Alshirifi, 2018). Other methods are dependent on the diazotization of 2,4-dinitroaniline (Hasan, et al., 2020), sulfacetamide sodium (Al-Uzri, 2019), p-nitroaniline (Ibraheem, 2009), clonazepam (Alteemi & Kadim, 2020), 2-aminothiazole (Mzban, et al., 2020), 2-aminobenzothiazole (Othman & Fatah, 2009) and coupling with PPH. Other dependent on the oxidation of PPH either with an excess of chloramine-T or bromosuccinamide (NBS) and the residual chloramine-T or NBS were estimated by bleaching the colour of indigo carmine dye (Battu, et al., 2020) and methyl orange dye (Zakaria, 2021), respectively. PPH was also determined through charge transfer complex formation with haematoxylin in an alkaline medium (Battu, et al., 2020). However, many of these methods suffer from various limitations, such as low sensitivity, low stability, time consuming and required solvent extraction, a number of manipulation steps, temperature control, and expensive devices that may not exist in the laboratory.

The present work is to proposes a simple and precise spectrophotometric method for assaying PPH as pure and in its pharmaceutical forms. The principle of this method was based on the oxidative coupling reaction between PPH and p-aminobenzophenone (p-ABph) using potassium periodate (KIO_4) as an oxidant to form a taupe-red product at room temperature.

2. Materials and Methods

Apparatuses

Devices of a Jasco V-630 digital double beam UV-Vis spectrophotometer equipped with 1.0-cm matched glass cells and Bp3001 professional bench top pH meter were used for all absorption spectra recording and pH measurements, respectively.

Chemical reagents and materials

The chemical materials and reagents used in this research displayed a considerably high degree of purity and were acquired from the BDH and Fluka companies.

PPH solution (100µg /mL):

It was prepared by weighing 0.0100g of PPH and dissolving it in suitable volume of distill water, and in a standard flask of 100mL, the volume is completed to the mark using DW.

p-ABph solution (0.1%):

A 0.250 g of p-ABP reagent was dissolved in a quantity of D.W and the volume was completed to 250 mL using standard flask.

Potassium periodate (0.25 %):

A 0.2500g of potassium periodate was set in 100mL D.W using a standard flask.

Pharmaceutical preparations

PPH injection (500 µg/10mL):

It was prepared by mixing five ampoules of PPH very well in 50 mL calibrated of volumetric flask, each mL of this solution containing 50 µg of PPH.

Nasofen drop (1%):

To obtain (100 µg/mL) of the PPH drug, 1 mL of 1% nasofen drop was transferred to a100-mL calibrated of volumetric flask and the volume was diluted to the mark with D.W.

Suggested procedure

An aliquot of a standard solution containing 0.2-30.0µg/mL of PPH was transferred into a sequence of 10 mL calibrated of volumetric flasks .To these solutions, 2.0 mL of potassium periodate (0.25%) was added followed by 2.0 mL of p-ABph (0.1%). The solutions were shaken thoroughly for 5.0 min to allow the coupling reaction to go to completion then the contents were diluted to the mark with D.W and mixed well. The absorbance of the taupe-red colour product was measured at 512 nm against a reagent blank which prepared similarly but without drug content.

3. Results and Discussion

Principle of the method

In this work PPH was treated with a suitable oxidizing agent and the resulting species was then coupled with p-ABph reagent in an aqueous medium to form an intensely coloured product.

Selection of the suitable oxidizing agent

The effect of 1mL volume of various oxidizing agents (0.2%) has been tested for oxidizing 100 µg/mL PPH. The results in Table 1 reveal that the reaction among PPH and p-ABph in the presence of potassium periodate as an oxidant is the best due to it's highest absorbance at 512. nm. Therefore, potassium periodate was chosen for the next experiments.

Table 1
Selection of the oxidizing agent

Type of oxidizing agents (0.2%)	Absorbance	λmax
Sodium periodate	0.1243	473.0
Potassium periodate	**0.1684**	**512.0**
N-Bromosuccinimide	0.0718	317.0
N-Chlolrosuccinimide	0.0059	487.0
Chloramine-T	0.0728	320.0

Influence of potassium periodate concentration

The influence of different concentrations (0.15- 0.35%) of potassium periodate was investigated on the absorbance of the resulting product. The results in Table 2 indicated that the 0.25% concentration of potassium periodate is the optimum, therefore, it has been selected for the following experiments.

Table 2
Effect of potassium periodate concentration on absorbance

KIO4 conc. (%)	0.15	0.17	0.2	0.25	0.3	0.35
Absorbance	0.2058	0.2106	0.2124	0.2190	0.2158	0.2065

The effect of several quantities (0.5- 4.0 mL) of potassium periodate has been also studied on the sensitivity of the resulting product. The results obtained in the Fig.2 revealed that 2.0 mL of 0.25% potassium periodate gives the highest sensitivity (A=0.2327) and this volume is recommended for the subsequent studies.

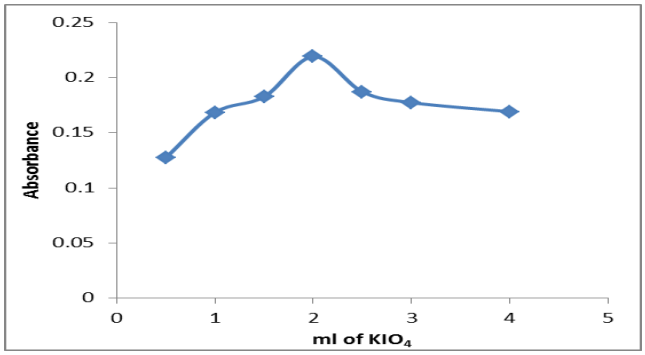
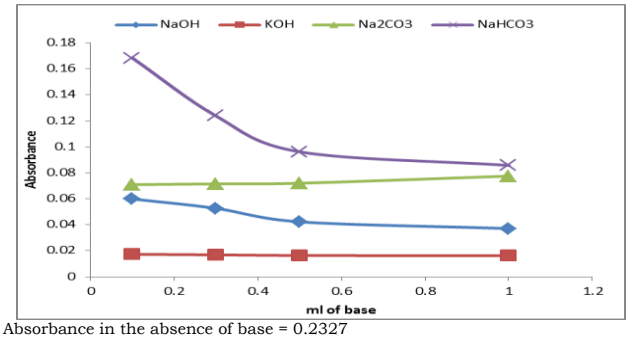


Fig. 2. Influence of 0.25% KIO4 amount on absorbance

Effect of basicity and acidity of the aqueous solution

The influence of several different quantities from 0.1 to 1.0 mL of various basic solutions [NaOH, Na_2CO_3, KOH, and $NaHCO_3$] (1M) and various acid solutions [HCl, HNO_3, H_2SO_4, CH_3COOH] (1M) were tested by using 10 mL calibrated of volumetric flasks for this purpose. The results are illustrated in Fig. 3 and 4.



Absorbance in the absence of base = 0.2327

Fig. 3. Influence of various bases and their quantities on absorbance

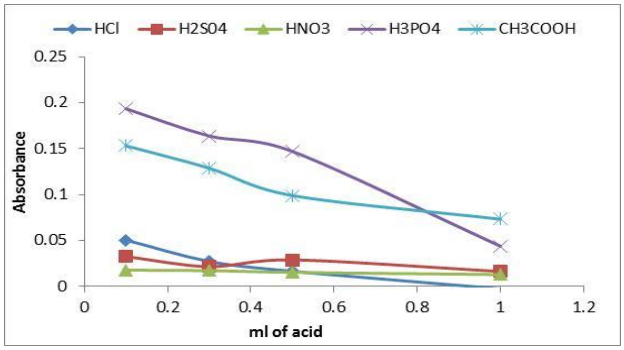


Fig. 4. influence of various acids and their quantities on absorbance

The results in Fig. 3 and 4 reveal that the addition of basic and acidic solutions caused decreasing in the absorption intensity of the product; therefore, they were excluded in the next experiments.

Effect of p-ABph reagent amount

The influence of several amounts from 1.0 to 3.0 mL of 0.1% p-ABph reagent on the absorbance of the coloured product was examined. The obtained results shown in Table 3 which indicate that the absorbance is increased with increasing the amount of p-ABph reagent until reaches the optimum by using 2.0 mL of p-ABph which gave a good (R^2 = 0.9989). Thus, the addition of 2.0 mL of p-ABph reagent was recommended for the subsequent experiments.

Table 3
Influence of p-ABph reagent amount on absorbance

mL of p-ABph (0.1%)	Absorbance / µg of PPH added in 10 mL					R2
	25	50	100	150	200	
1.0	0.0574	0.1058	0.2325	0.3485	0.4299	0.9945
1.5	0.0612	0.1171	0.2412	0.3544	0.4431	0.9964
2.0	0.0635	0.1252	0.2673	0.4132	0.5331	0.9989
2.5	0.0556	0.1152	0.2478	0.3943	0.4943	0.9962
3.0	0.0525	0.1025	0.2221	0.3183	0.4064	0.9966

Sequence of addition

The influence of the addition order of the reactants on the colour development was also examined. Maximum absorbance (0.2776) was achieved by performing the following order of addition (PPH + potassium periodate + p-ABph)

Influence of temperature and oxidation time

For the purpose of showing the effect of temperature on the absorbance of the resulting product, the oxidation reaction of PPH with potassium periodate was carried out at three different temperatures (5, room temperature and 50°C) using a thermostatic water bath at different periods of times before the addition of p-ABph reagent. The results in Fig.5 are display that the oxidation of PPH with potassium periodate occurred immediately at room temperature (RT= 23±2).

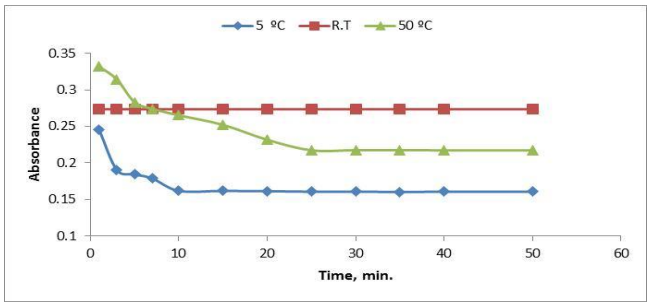


Fig. 5. Effect of temperature and oxidation time on absorbance

The effect of time on the colour development of the resulting product was also carried out by measuring the absorbance of the final solution at different periods of time. The experimental results in Fig.6 revealed that the colour of the resulting product gives highest absorbance after 5.0 minutes and no noticeable change was appeared on the absorbance for at least 60.0 minutes at room temperature

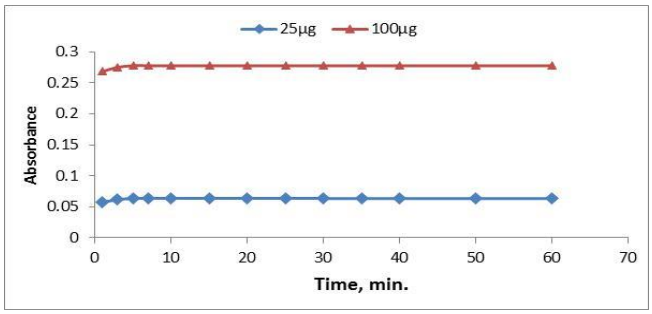


Fig. 6. Effect of time on the colour development of the product

Spectral characteristics

This work involves the oxidation of PPH by potassium periodate then coupling with p-ABph and forming a taupe-red colour product having a maximum absorption at 512 nm. Which was used for all subsequent measurements. The absorption of the reaction product is shown in Fig 7.

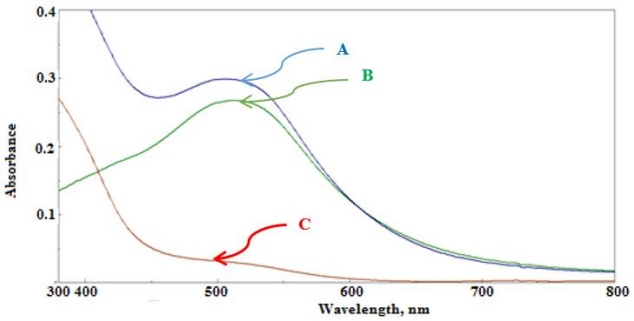


Fig. 7. The absorption spectra of 100 µg/mL PPH measured at A, B and C against D.W, reagent blank, and blank against D.W, respectively

Reproducibility and Validity of Beer's law

By utilizing the best conditions of recommended procedure (λmax=512nm.) a linear calibration graph was constructed in the concentration range 2.0-20 µg/mL of PPH with determination coefficient (R^2=0.9986) (Fig.8). The molar absorptivity and the index of Sandell's sensitivity were tested and evenness to 0.552×10^4 L/mol.cm. and 0.0368 µg/cm^2, respectively. The detection limit (DL) and quantification limit (QL) were estimated and equal to 0.0094 and 0.0313 µg/mL, respectively (Nejres & Najem, 2023). Accuracy (relative error %) and precision (RSD) of the present method were worked out via estimating five replicate

of phenylephrine solution at three concentration levels and found to be in the range -0.0479% to -0.0145% and 0.0715 to 0.0216, respectively.

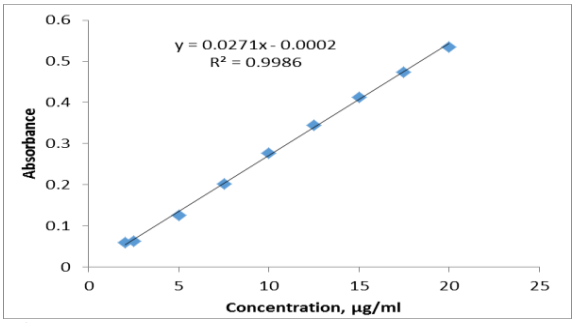


Fig. 8. Calibration graph for the determination PPH

Stoichiometry of the resulting product

The composition of the reaction between the oxidized PPH and the organic reagent p-ABph was investigated by using the Job's and mole ratio methods (De Levie, 1997). In Job's method, volumes 0.25–1.75mL of 4.910×10^{-4} M of oxidized PPH were coupled according to the recommended procedure with the corresponding complementary volume of 4.910×10^{-4} M p-ABph solution to give a total volume of 2 mL and diluted to 10 mL with D.W. While in mole ratio method, increased volumes 0.25–2.5 mL of 4.910×10^{-4} M p-ABph solution were added to 1.0 mL of 4.910×10^{-4}M of oxidized PPH and the absorbance was recorded at 512 nm after dilution to the mark with D.W. The results obtained in Fig. 8 are illustrated that the resulting product was formed by a 1:1 combining ratio of oxidized PPH to p-ABph reagent. The stability constant value of the product was also evaluated and it was equivalent to 6.08×10^{6} L/mol (Miller & Miller, 2018).

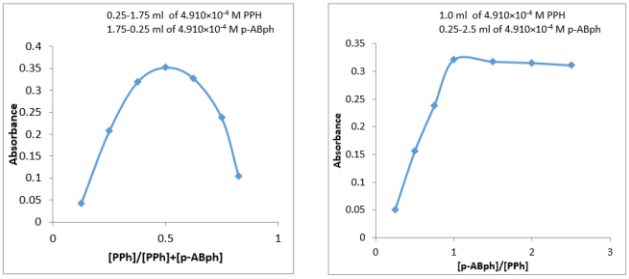


Fig.9: (a) Job's method plot and (b) Mole ratio method plot

Equations of the reaction

A mechanism of interaction has been suggested which includes PPH oxidation using potassium periodate as an oxidizing agent, and the conjugation of the product formed to give a product of a taupe-red colour measured at a wavelength 512.0 nm, as shown by the equations below:

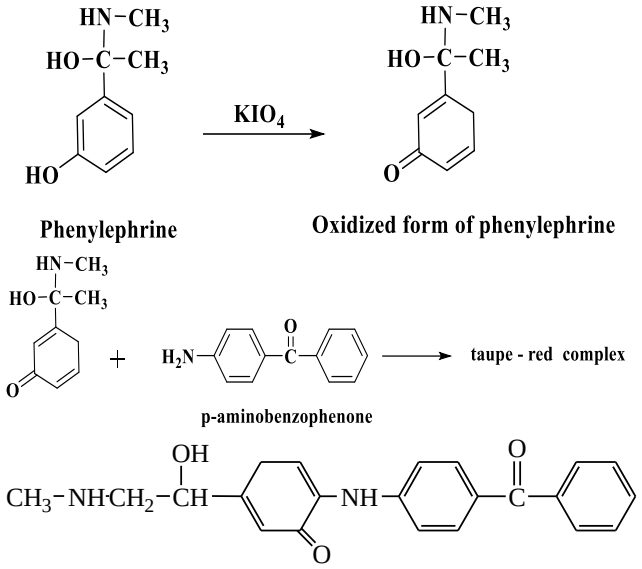


Fig. 10. Taupe–red colour product

Applications

To investigate the ability of the suggested method, the content of phenylephrine in the commercial pharmaceuticals (injection and drop) at three different quantities (50, 100, 150 µg) was estimated under the recommended procedure. The results listed in Table 4 indicate that the proposed procedure is in good agreement with the declared content and there is no important variance in relative error and relative standard deviation.

To evaluate the results of the recommended method a t-test has been carried out. The results in Table 4 are reveal that the values of t-experimental were under the artificial value (t=2.776) at 95% confidence level and for four degrees of freedom (N=4) (Christian, et al., 2013). The results indicated that the difference were statistically not significant, which confirms the success of applying the proposed method to estimate PPH in its drugs.

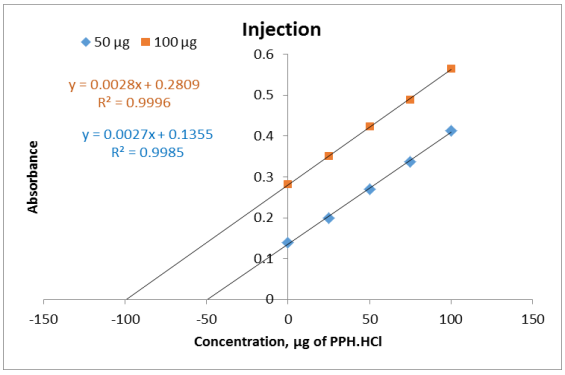
Table 4
Determination of PPH in injection and Nasofen drop

Pharmaceutical preparation	Certified value	PPH* found(µg)	R.E* (%)	Recovery (%)	RSD* (%)	Measured value*	t-exp
Phenylephrine. HCl injection (France)	500 µg /10 mL	49.99	-0.02	99.98	0.0357	499.90 µg	1.00
		99.99	-0.01	99.99	0.0161	499.95 µg	1.24
		150.01	0.00	100.00	0.0108	500.00 µg	1.00
Nasofen drop 1% (Pioneer-Iraq)	1%	49.984	-0.03	99.97	0.0437	0.9997 %	1.63
		99.98	-0.02	99.98	0.0197	0.9998 %	1.94
		149.99	-0.01	99.99	0.0108	0.9999 %	1.24

* Average of five estimations, t-exp.: t-experimental, $t \pm = (\bar{x} - \mu)\frac{\sqrt{N}}{S}$

Standard Additions Method

To evaluate the efficiency of the proposed method for the determination of phenylephrine and its good ability in application in pharmaceuticals (injection and drop), standard addition method was applied and it gave results get along well with the proposed method. The results are explained in Fig.11 and tabulated in Table 5 indicating that the standard addition method is in agreement with the present method. The current is within the acceptable range of the error, which reveals the possibility to applying the current method on pharmaceuticals.



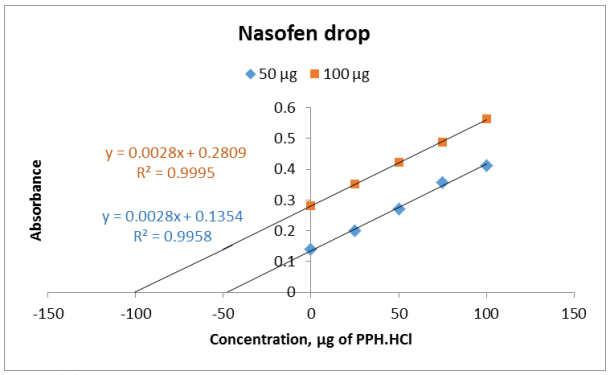


Fig. 11. Curves of Standard addition method for the determination of PPH in injection and Nasofen drop

Table 5
Analysis of PPH in injection and nasofen drop by standard additions method

Pharmaceutical preparations	PPH present(µg)	PPH found (µg)	Recovery (%)	Relative error (%)
Phenylephrine.HCl injection 500 µg /10mL (France)	50	50.18	100.36	0.36
	100	100.32	100.32	0.32
Nasofen drop (1%) (Pioneer- Iraq)	50	50.15	100.3	0.3
	100	100.32	100.32	0.32

4. Conclusions

This work describes a simple, accurate and sensitive spectrophotometric method for the estimation of PPH in pure form and in some pharmaceutical forms through oxidative coupling reaction. The method does not need solvent extraction steps or temperature control. The method is also precise and selective to be successfully applied for estimating PPH in injection, Nasofen drops.

Competing Interests

The authors have declared that no competing interests exist.

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