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Research Article

NEW SPECTROPHOTOMETRIC METHODS DEVELOPMENT FOR THE DETERMINATION OF OSELTAMIVIR PHOSPHATE IN CAPSULES BASED ON THE OXIDATION REACTIONS OF THE OLEFENIC DOUBLE BOND

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ABSTRACT

Simple, sensitive and selective spectrophotometric methods (M_1 and M_2) for the assay of oseltamivir phosphate (OP) through the olefenic double bond are proposed. Method M_1 is based on the reaction of potassium permanganate to the olefenic double bond in OP and estimating the unreacted permanganate with fast green FCF (FGFCF). Method M_2 involves the treatment of the olefenic double bond in OP with a Lemieux reagent (mixture of $KMnO_4$ and $NaIO_4$) and estimating the aldehyde formed with 3-methyl-2-benzothiazolinone hydrazone (MBTH). The color produced in M_1 and M_2 methods has maximum absorption at 620nm and 654nm respectively. Beer's law obeyed in the concentration range of 4-20µg/ml and 4-12 µg/ml for method M_1 and M_2 respectively. The precision and accuracy of the methods are checked by the reported UV reference method. No interference was observed from the usually existing additives in pharmaceutical formulations and the applicability of the methods was examined by analyzing NATFLUE capsules containing OP. The both methods are found to be suitable for the determination of oseltamivir phosphate.

KEY WORDS: Assay, FGFCF, Lemieux reagent, MBTH, Beer's law

INTRODUCTION

Oseltamivir phosphate (OP) (Fig.1) is the best known orally active newest addition to the group of H_1N_1 and H_5N_1 neuraminidase inhibitor and an antiviral drug that slows the spread of influenza (flu) viruses (type A and B) between cells in the body by stopping the new virus from chemically cutting ties with its host cell. The drug is considered the best treatment for the bird flu disease. OP is an ethyl ester pro-drug that is rapidly and extensively metabolized by esterases in the gastrointestinal tract and liver to its active form, oseltamivir carboxylate(OC). OP is a white crystalline powder solid with the chemical name (3R,4R,5S)-4-acetylamino-5-amino-3(1-ethylpropoxy)-1-cyclohexene-1-carboxylicacid,ethylester, phosphate (1:1) and Its chemical formula is $C_{16}H_{28}N_2O_4.H_3PO_4$ representing molecular weight of 410.4.

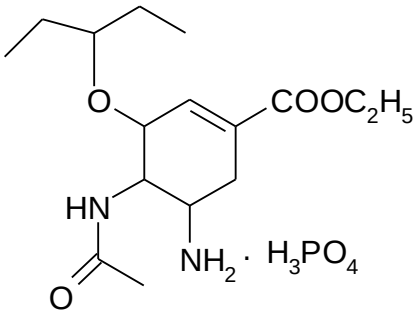

Fig.1: Chemical structure of OP

In literature, OP can be identified by thin layer chromatography, specific optical rotation, infrared spectrophotometry and tests characteristic for ortho phosphates[1], Determination, by International Pharmacopeia [2], can be done by high-performance liquid chromatography [1-2] or by titration with perchloric acid [1]. Other analytical methods such as UV spectroscopy [3-5], visible spectrophotometric [6-9], colorimetric and LC[10], spectrofluorimetric [11], HPLC with UV detection [12-19] and mass spectrometry[20-23], Micellar electrokinetic chromatography[24], capillary electrophoresis[25] voltammetry[26] and potentiometry [27] have been reported for the determination of OP in biological fluids and formulations. In the current scenario for the analysis of drugs many oxidants have been applied for oxidation. Very few workers have used oxidants to exploit olefenic double bonds. This paper describes analytical studies on the role of oxidants in the olefenic double bond in oseltamivir phosphate. It is well known that compounds of the R-CH=CH-R^1 type undergo oxidation with acid permanganate, directly yielding a mixture of carboxylic acids , while in the presence of sodium metaperiodate(Lemieux reagent) they yield a mixture of aldehydes. Existing analytical methods reveal that relatively little attention has been paid to develop visible spectrophotometric methods. The low λ_{max} value of the colored species in many of the reported methods prompted us to explore the possibility of developing new methods with a higher λ_{max}. The efforts of this accord resulted in two such procedures, based on the oxidation of OP with $KMnO_4$ and a Lemieux reagent and estimating the unreacted permanganate with FGFCF [28](Method M_1) or the aldehyde formed with MBTH [29] (Method M_2). The results of these methods are statistically validated. These methods can be extended for the routine quality control analysis of pharmaceutical products containing OP.

MATERIALS & METHODS (EXPERIMENTAL)

Apparatus and chemicals

A Milton Roy UV/Visible spectrophotometer model-1201 with 10mm matched quartz cells was used for all spectral measurements. All the chemicals used were of analytical grade and the solutions wee freshly prepared. Aqueous solutions of acid $KMnO_4$ (BDH, 0.0316%, $2.0x10^{-3}$M for M_1 or 0.01% $6.32x10^{-4}$M for M_2 in 2.0M H_2SO_4), FGFCF solution (Chroma, 0.01%, $1.23x10^{-4}$M prepared by dissolving 10mg of fast green FCF in 100ml of 1.0M sulphuric acid. 10ml of this solution was further diluted to 100ml with the same strength of acid), Na_2SO_4 solution (BDH,4.2%, 1.0M, prepared by dissolving 4.2g of sodium sulphate in 100ml of distilled water), $NaIO_4$ (Qualigens, 0.05%, $2.33x10^{-3}$M , prepared by dissolving 50mg of sodium meta periodate in 100ml of distilled water and MBTH (Fluka, 0.2%, $8.55x10^{-3}$M, prepared by dissolving 200mg of MBTH in 100ml of distilled water) were used.

Preparation of standard drug solution: A 1mg/mL solution was prepared by dissolving 100mg of oseltamavir phosphate in 100ml of 20% acetic acid and the stock solution was diluted stepwise with distilled water to obtain working standard solutions of 100µg/mL for the both methods (M_1 and M_2).

Analytical Procedures:

Determination of wavelength maximum (λ_{max}):

Method M_1:

Method M_1: 5.0ml of Standard OP solution was transferred into 25ml calibrated tube. To this 0.5ml of $KMnO_4$ ($2.063x10^{-3}$M) solution was added. And the total volume in tube was brought to 10ml with distilled water and set aside for 10 min at laboratory temperature. Then 4.0ml each of the FGFCF ($1.236x10^{-4}$M) solution and sodium sulphate (1.0M) solution were added successively After 10 min, the volume was made up to the mark with distilled water. In order to investigate the wavelength maximum, the above colored solution was scanned in the range of 400-660 nm UV-Visible spectrophotometers against a reagent blank. From the absorption spectra (Fig.2), it was concluded that 620nm is the most appropriate wavelength for analyzing OP with suitable sensitivity.

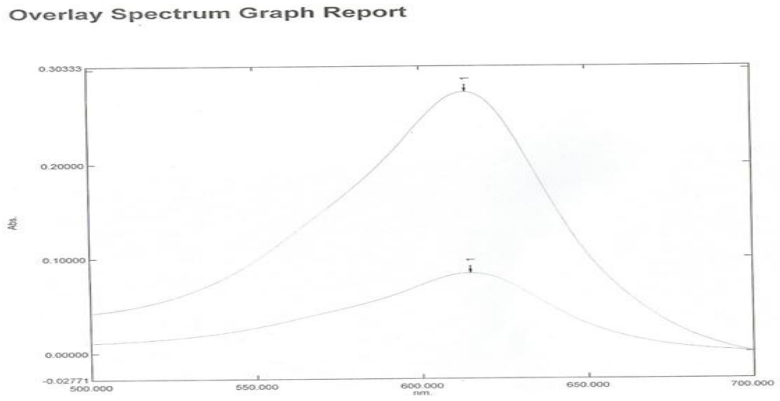

Fig.2: Absorption spectra of OP-$KMnO_4$-FGFCF system

Method M_2: 3.0 ml of Standard OP solution was transferred into 25ml calibrated tube. Then 0.5mL of $KMnO_4$ ($6.32x10^{-4}$M) and 1.0mL of $NaIO_4$ ($2.33x10^{-3}$M) solutions were added successively and kept in a boiling water bath for 10 min. After that 1.0mL of MBTH ($8.56x10^{-3}$ M) solution was added and heated for another 3 min. The solution was cooled to room temperature and the total volume in tube was made up to the mark with distilled water. In order to investigate the wavelength maximum, the above colored solution was scanned in the range of 400-660 nm UV-Visible spectrophotometers against a reagent blank. From the absorption spectra (Fig.3), it was concluded that 654nm is the most appropriate wavelength for analyzing OP with suitable sensitivity.

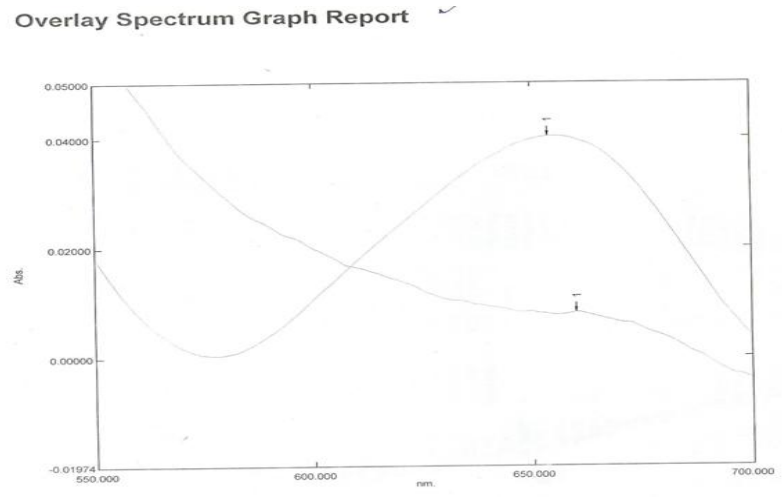

Fig.3: Absorption spectra of OP-$NaIO_4$-MBTH

Preparation of calibration curve:

Method M_1: Aliquots of Standard OP solution (1.0-5.0mL, 100µg/mL) were transferred into a series of 25mL calibrated tubes. To each tube 0.5mL of $KMnO_4$ ($2.063x10^{-3}$M) solution was added and the total volume in each tube was brought to10mL with distilled water and set aside for 10 min at laboratory temperature. Then 4.0mL each of the FGFCF solution and sodium sulphate solution were added successively and set aside for 5 min. for complete color development and then diluted to the mark with distilled water. The absorbance was measured at 620nm against a reagent blank prepared simultaneously. The decrease in absorbance corresponding to the drug content was obtained by subtracting the absorbance of the blank from that of test solution. The amount of drug was computed from its calibration graph (Fig.4).

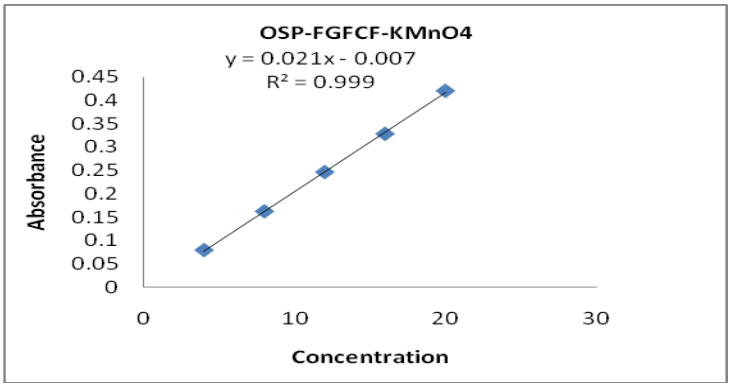

Fig.4: Beer's law plot of OP-$KMnO_4$-FGFCF system

Method M_2:

Aliquots of Standard OP solution (1.0-3.0mL, 100µg/mL) were transferred into a series of 25mL calibrated tubes. Then 0.5mL of $KMnO_4$ ($6.32x10^{-4}$M) and 1.0mL of $NaIO_4$ ($2.33x10^{-3}$M) solutions were added successively and kept in a boiling water bath for 10 min. After that 1.0mL of MBTH ($8.56x10^{-3}$ M) solution was added and heated for another 3 min. The solution was cooled to room temperature and the total volume in each tube was made up to the mark with distilled water. The absorbance was measured after 5 minutes before 60minutes at 654nm against the reagent blank prepared similarly. The content of the drug computed from the appropriate calibration graph (Fig.5).

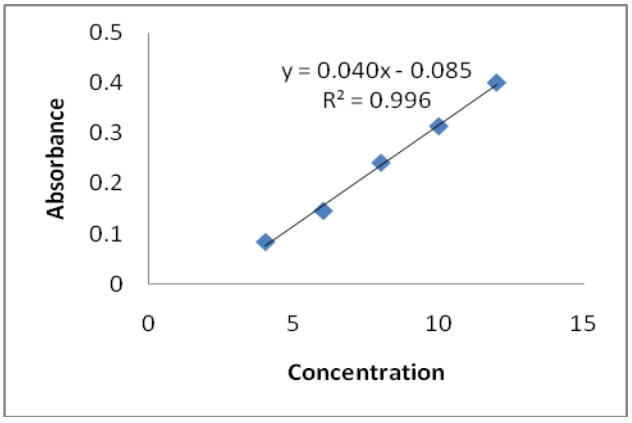

Fig.5: Beer's law plot of OP-$NaIO_4$-MBTH syste

For pharmaceutical formulations:

Preparation of Sample solution

About 10 capsules were weighed to get the average weight and pulverized and the powder equivalent to 100mg of OP was weighed, dispersed in 25ml of isopropyl alcohol (IPA), sonicated for 30minutes and filtered through whatman filter paper no.41. The filtrate was evaporated and the residue was used for the preparation of working sample solution in the same way as under working standard solutions and analyzed under the procedures for the bulk samples. The UV method reported earlier using 0.1M NaOH (λ_{max} =216nm) as a solvent was chosen as the reference method for ascertaining the accuracy of the proposed methods.

RESULTS AND DISCUSSION

The working conditions for the color developments of methods M_1 and M_2 were established by varying the parameters on at a time and keeping the others fixed and observing the effect produced on the absorbance of the colored species. The following experiments were conducted for this purposed and the conditions so obtained were incorporated into the recommended procedures. **Method M_1**: 0.4 to 0.6ml of $KMnO_4$ ($2.063x10^{-3}$M) and a waiting time of 5 to 15 min at room temperature were found to be adequate. A prolonged waiting period or an increase in temperature has no additional advantage. Hence 0.5ml of $KMnO_4$ and a waiting time of 10 min were preferred. To maintain the linear relationship between the un-reacted $KMnO_4$ and FGFCF, the addition of 4.0ml each of $1.23x10^{-4}$M and 1.0M sodium sulphate were found to be optimum. The consistency in absorbance after the gradual decrease of FGFCF was attained within 5 min and remained stable for further 45 min, and was measured at 620nm.

Method M_2: In the first step, 0.4 to 0.6ml mL of $6.32x10^{-4}$M $KMnO_4$ and 0.5to 1.5mL of ($2.33x10^{-3}$M) $NaIO_4$ and heating on a boiling water bath for 10to20 min. were found to be necessary to get constant and reproducible absorbance values. The values were erratic beyond this range. In the second step, an optimum range of 0.5 to 1.5mL of MBTH ($8.56x10^{-3}$ M) and further heating on a boiling water bath for 2 to5min were found to be adequate to get the maximum absorbance. Thus list volumes of $KMnO_4$ (0.5mL, $6.32x10^{-4}$M), $NaIO_4$(1.0mL, $2.33x10^{-3}$M) and MBTH(1.0mL, $8.56x10^{-3}$ M) and heating times of 10 before and 3 min after the addition of MBTH were preferred .The color product was stable for one hour and was measured at 654nm.

Analytical Data

In order to test whether the colored species formed in the methods adhere to Beer's law, absorbances at appropriate lengths of a set of solutions containing varying amounts of OP and specified amounts of reagents(as given the recommended procedures for each method) were recorded against the corresponding reagent blank. The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements containing $3/4^{th}$ of the amount of the upper Beer's law limits), Regression characteristics like standard deviation of slope (S_b), standard deviation of intercept (S_a), standard error of estimation (S_e) and % range of error (0.05 and 0.01 confidence limits) were calculated and the results are summarized in Table-1.Nat flu capsules were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically by the t-and F-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre-analyzed formulations at three different concentration levels. MS Excel Software-2007 used for calculations and graphs. These results are summarized in Table-2.

Table - 1 Optical characteristics, precision and accuracy of the proposed methods

Parameters	Method M_1	Method M_2
λ_{max}(nm)	620	654
Beer's law limit (µg/ml)	4- 20	4-12
Sandell's sensitivity (µg/cm^2/0.001 abs. unit)	0.00195122	0.001327801
Molar absorptivity (Litre/mole/cm)	210330	309082.5
Regression equation (Y) *= a +b x		
Intercept (a)	-0.007	-0.085
Slope(b)	0.021	0.040
%RSD	1.2	1.89
% Range of errors(95% Confidence limits)	1.26	1.99
0.05 significance level	1.98	3.12
0.01 significance level		

*Y = a + b x, where Y is the absorbance and x is the concentration of OP in µg/ml

Table-2 Analysis of OP in pharmaceutical formulations

Method	*Formulations	Labeled Amount (mg)	Found by Proposed Methods			Found by Reference Method ± SD	#% Recovery by Proposed Method ± SD
			**Amount found ± SD	t	F		
M_1	Capsule-1	30	29.59±0.32	1.64	3.94	29.80±0.16	98.62±1.07
	Capsule-2	75	74.44±0.49	0.54	3.35	74.65±0.91	99.26±0.66
M_2	Capsule-1	30	29.79±0.13	0.35	1.55	29.80±0.16	99.29 ± 0.43
	Capsule-2	75	74.49 ±0.46	0.41	3.8	74.65±0.91	99.30 ± 0.62

* Capsule- 1 and capsule-2: Natflu capsules of NATCO PHARMA LIMITED, Hyderabad (India)

**Average ± Standard deviation of six determinations, the t- and f-values refer to comparison of the proposed method with UV reference method. Theoretical values at 95% confidence limits t =2.57 and F = 5.05.

Recovery of 10mg added to the pre analyzed sample (average of three determinations).

Reference method (reported UV method) using 0.1M NaOH (λ_{max}=216nm).

Chemistry of the color species:

Method M_1: This method was based on the reaction of permanganate to the olefenic double bond of cyclo hexene moiety in OP (first step) and an estimation of the un-reacted permanganate with FGFCF (second step). The probable sequence of reactions are presented in the scheme (Fig.6)

Method M_2: This method involves the treatment of an olefenic double bond with Lemieux reagent (first step) and an estimation of the aldehyde formed with MBTH in the presence of oxidants (excess permanganate and periodate left after the completion of the reaction) based on the formation of a brilliant blue cationic dye(second step). The probable sequence of reactions are presented in the scheme (Fig.7)

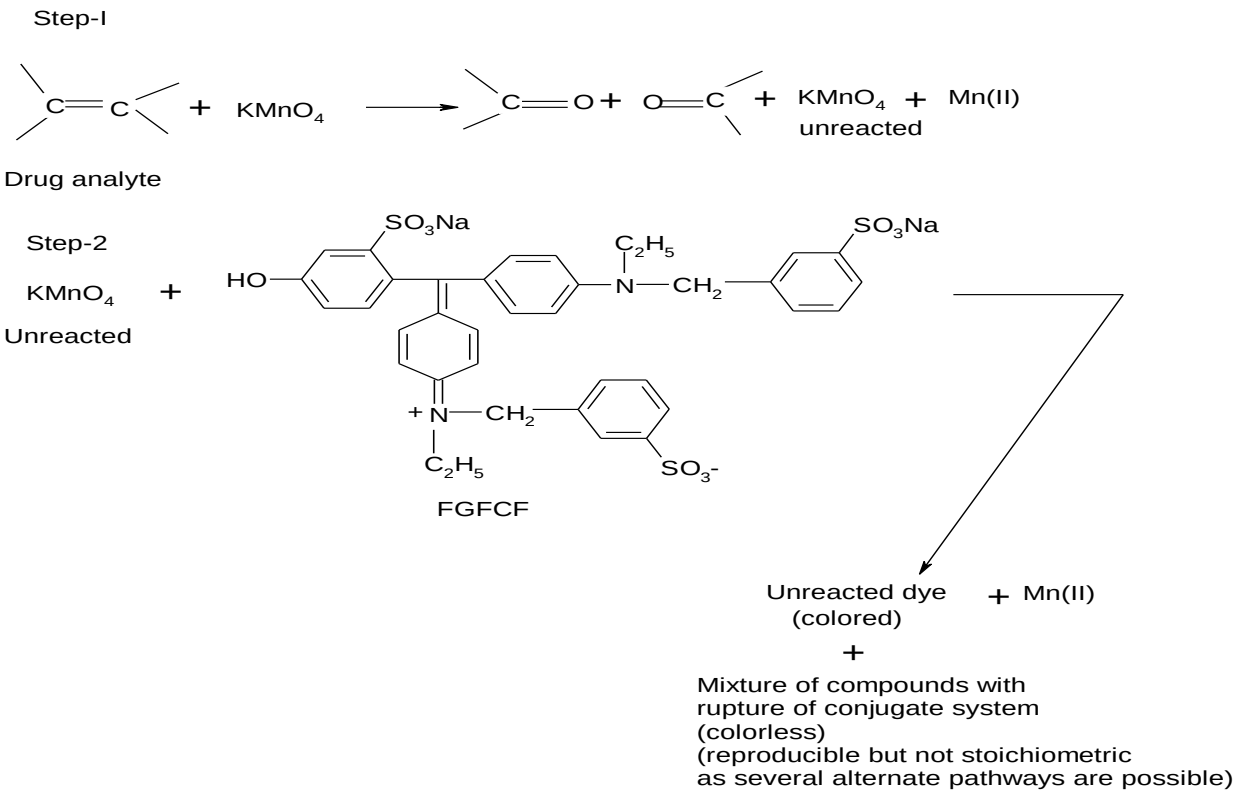

Fig.6: Probable scheme of the reaction for Method M_1

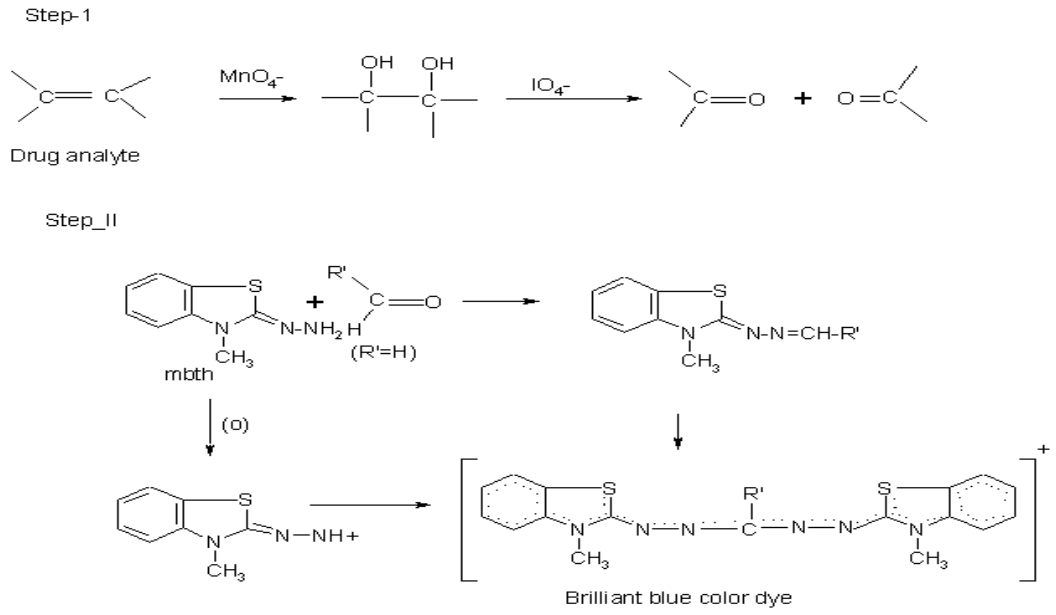

Fig.7: Probable Scheme of the reaction for Method M_2

CONCLUSIONS

The proposed methods are attractive ones compared to the reported methods since the proposed methods have higher λ_{max}. The contaminants do not interfere in the color development. This was further proven by the resulting percentage recoveries of the proposed methods. Hence the proposed methods are simple selective and reliable and can be used for the assay of OP in bulk samples and pharmaceutical formulations. These methods can be used as alternative methods to the reported ones.

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