UV Spectrophotometric Methods to Quantify Alogliptin Benzoate and Pioglitazone Hydrochloride

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Three new, precise, accurate and sensitive UV-Spectrophotometric methods namely Ratio Difference Spectroscopic Method (RDSM), First Derivative of Ratio Spectra Method (DR1) and Area Under Curve Method (AUC) were developed and validated for simultaneous assessment of alogliptin benzoate (ALO) and pioglitazone hydrochloride (PIO) in tablet dosage form. In RDSM, ratio spectra of both the drugs were recorded by dividing the mixtures using interfering drug as divisor. Then the difference between the amplitudes of obtained ratio spectra was measured at 288 and 291 nm for ALO and 236 and 245 nm for PIO. The second method DR1, where the first derivative of ratio spectra of both the drugs were recorded and the first derivative signal was measured at 290 nm for ALO and 276.8 nm for PIO. The scaling factor was fixed as 1 and wavelength interval (Δλ) as 2 for recording the first derivative of ratio spectra. In the third method (AUC), peak area of recorded zero order spectra was measured at 276 ± 10 nm for ALO and 267.8 ± 10 nm for PIO. All three proposed methods were validated according to “International Conference on Harmonization” (ICH) guidelines parameters. For all three methods, ALO and PIO obeyed Beer’s law in the range of 0.5-5 & 1.8-18 µg/ml, respectively. The % RSD of repeatability of measurement, intra-day and inter-day precision were found to be less than 2 for all three methods.
Limit of detection (LOD) and Limit of quantification (LOQ) of the drugs were calculated which proved the sensitivity of the methods. The accuracy ranged between 98-101% for all three methods. No interference from pharmaceutical excipients present in the formulation was observed. These proposed methods were found to be simple, sensitive, accurate and precise and can be applied to the simultaneous estimation of ALO and PIO in combined tablet formulation and also appropriate for routine quality control analysis.

Keywords: Alogliptin benzoate; pioglitazone hydrochloride; ratio difference spectroscopic method; first derivative of ratio spectra method; area under curve method; laboratory prepared tablet formulation.

1. INTRODUCTION

The incidence of type 2 diabetes is at epidemic proportions throughout the world. Patients with diabetes have a 2–4-fold increased risk of cardiovascular disease when compared to the general population. They also have a greatly increased risk for microvascular disease. Hence, medications that successfully control hyperglycemia in type 2 diabetes patients are of utmost importance [1-2]. Alogliptin benzoate (ALO) is a dipeptidyl peptidase inhibitor with a chemical designation of 2-[(6-[(3R)-3aminopiperidin-1-y]]-3-methyl-2, 4-dioxo-3,4-dihydropyrimidin-1(2H)yl]methyl) benzonitrile (Fig. 1). ALO appears as crystalline powder which is white to off-white in color and it is soluble in dimethyl sulfoxide, sparingly soluble in methanol and water, marginally soluble in ethanol and very slightly soluble in isopropyl acetate and octanol [3]. ALO act by prolonging the action of incretins, the gut hormones, thereby increasing bloodstream concentrations and reducing fasting and postprandial glucose concentrations in a glucose dependent manner in patients with type 2 diabetes mellitus. The inhibition of DPP-4 increases the amount of active plasma incretins which helps with glycemic control and boost insulin levels. ALO is a potent, highly selective dipeptidyl peptidase-4 inhibitor which is effective and well tolerated as a treatment for type 2 diabetes, either as monotherapy or in combination with metformin, thiazolidinediones, sulfonylureas and insulin, with an excellent safety profile [4].

The hydrochloride salt, IUPAC named as (±)-5-[[4-2-(5-ethyl-2-pyridinyl) ethoxy] phenyl] methyl]-2, 4-thiazolidinedione monohydrochloride (PIO) (Fig. 1) is appears as crystalline white powder which is odorless. It is soluble in N, N dimethyl formamide and methanol, marginally soluble in ethanol, very slightly soluble in acetonitrile and acetone, insoluble in ether and water [3]. PIO is a synthetic ligand for PPAR peroxisome proliferator-activated receptor – (PPARγ) mainly present in adipose tissue, muscles and liver. It alters transcription of gene products that are important in insulin signaling, including lipoprotein lipase, fatty acid transporter protein, adipocyte fatty acid-binding protein, glucose transporter 1 and 4. Thus, PIO is an oral antihyperglycemic drug. PIO improves glycemic control in people with type 2 diabetes by improving insulin sensitivity through its action at PPARγ-1 and PPARγ-2, and affects the lipid metabolism through action at PPAR alpha. PIO is generally well tolerated, weight gain and oedema are the most common emergent adverse events, and there are no known drug interactions between PIO and other drugs. In clinical trials in patients with type 2 diabetes mellitus, PIO as monotherapy, or in combination with metformin, repaglinide, insulin or a sulphonylurea, induced both long- and short-term improvements in glycemic control and serum lipid profiles. PIO was also effective in reducing some measures of cardiovascular risk and arteriosclerosis. PIO thus offers an effective treatment option for the management of patients with type 2 diabetes [5-6]. Studies indicate that combination treatment with ALO and PIO at an early stage of diabetes improved metabolic profiles and indices that measure beta-cell function, compared with either ALO or PIO monotherapy [7-8]. A thorough literature review revealed several methods for the estimation of ALO & PIO, individually or in combination with other drugs. A few estimations in single and in combined tablet dosage forms have been reported for ALO and PIO such as UV Spectrophotometry [9-12], HPLC [13-14] and HPTLC [15-16]. Some other analytical procedures are also available in the literature for the analysis of PIO with other drugs using high performance liquid chromatography. However, the reported UV-spectrophotometric estimations lacks in sensitivity and concentration range are not in proportion as they are present in the marketed formulation. Hence, in this manuscript tried to develop and validate some more...
sensitive, simpler, precise, accurate and cost-effective UV spectroscopic methods for the simultaneous determination of ALO and PIO in combined tablet formulation which can be used as an alternative to the reported methods. So, the present paper describes three UV spectroscopic methods namely Ratio Difference Spectroscopic Method (RDSM), First Derivative of Ratio Spectra Method (DR) and Area Under Curve Method (AUC) for the simultaneous estimation of ALO and PIO in tablet dosage form.

2. MATERIALS AND METHODS

2.1 Materials, Chemicals and Reagents

ALO was purchased from Swapnroop Drugs and Pharmaceuticals, Aurangabad, Maharashtra, India and PIO reference standard was received as gift sample from Cadila Healthcare, Ahmedabad, India. In-house Laboratory manufactured tablet formulation containing 12.5 mg of ALO and 45 mg of PIO was utilized for the research work. Methanol (AR Grade) was purchased from SD Fine Chemicals, Mumbai, India.

2.2 Instruments Used

Shimadzu double beam UV-visible spectrophotometer (UV-1800, UV Probe, Shimadzu Corporation, Kyoto, Japan) with matched quartz cell of 1 cm path length was used for the analysis. All weighing was performed on highly sensitive Adventurer-Pro, AVG264C electronic balance, Ohaus Corporation, Pine Brook, NJ, USA.

2.3 Preparation of Solutions

2.3.1 Reference stock solutions of ALO and PIO

For the preparation of standard stock solution of ALO and PIO, 6.80 mg of ALO (6.80 mg of alogliptin benzoate is equivalent to 5 mg of alogliptin) and 18 mg of PIO (19.84 mg of pioglitazone hydrochloride is equivalent to 18 mg of pioglitazone) standard drugs were accurately weighed and transferred to 10 ml volumetric flasks separately. This solution was further diluted to 10 ml with methanol to obtain the concentration of the drugs, 500 and 1800 µg/ml, respectively. Further dilutions were made to get the desired concentration with methanol and overlain spectra were recorded (Fig. 2).

2.3.2 Reference stock solutions of ALO and PIO Mixture

Mixed standard solutions were prepared by mixing ALO and PIO standard solution in a series of 10 volumetric flask and volume was made up to the mark with methanol. Further dilutions were made to get the desired concentration with methanol.
Fig. 2. Overlain zero order absorption spectra of ALO (4 µg/ml) and PIO (14.4 µg/ml) using methanol

2.3.3 Preparation of sample solution

20 combined tablet formulations (Laboratory manufactured tablet containing 12.5 mg of ALO and 45 mg of PIO) was accurately weighed and crushed. Subsequently fine powder equivalent to 5 mg of ALO & 18 mg of PIO was transferred to a 50 ml volumetric flask (100 µg/ml ALO & 360 µg/ml PIO). 30 ml of methanol was added to the flask, vortexed and shaken for 10 minutes. Then the volume was made up to the mark with methanol and filtered through whatman filter paper no 41. The resulting solution was further diluted to get 3 µg/ml ALO &10.8 µg/ml PIO, respectively.

2.4 Procedures

2.4.1 Method 1: Ratio difference spectrophotometric method (RDSM) [17-22]

In RDSM, the absorption spectra of the prepared solutions (ALO: 0.5-5 µg/ml; PIO: 1.8-18 µg/ml; MIX: ALO: 0.5-5 µg/ml & PIO: 1.8-18 µg/ml) were scanned (200–400 nm) and stored in the computer. The stored spectra were divided by the absorption spectra of 0.5 µg/ml ALO and 14.4 µg/ml of PIO, respectively, where the obtained ratio spectra were recorded. Calibration curve was constructed for ALO by plotting the difference between the amplitudes of obtained ratio spectra at 288 nm and 291 nm and for PIO by plotting the difference between the amplitudes of obtained ratio spectra at 236 nm and 245 nm against the corresponding concentrations and the regression equations were computed.

2.4.2 Method 2: First derivative of ratio spectra method (DR1) [23-24]

In the DR1, the previously scanned zero order absorption spectrum of the MIX (ALO: 0.5-5 µg/ml & PIO: 1.8-18 µg/ml) were divided by the spectrum of ALO (0.5 µg/ml) and by spectrum of PIO (14.4 µg/ml) separately to get the ratio spectra of ALO and PIO, respectively. The first derivatives of the ratio spectra were then calculated. The amount of PIO was determined by measuring the first derivative signal at 276.8 nm. A similar procedure was followed for ALO at 290 nm. Amplitudes obtained from the first derivative signal were plotted against corresponding concentration and regression equations were computed.

2.4.3 Method 3: Area under curve method (AUC) [25]

In AUC, the absorption spectra of the prepared solutions (ALO: 0.5-5 µg/ml; PIO: 1.8-18 µg/ml) were scanned (200–400 nm) and stored in the
2.4.4 Analysis of sample solution

After scanning the sample solution (mentioned in Preparation of Sample Solution section) between 200 to 400 nm, zero order spectra were recorded. In RDSM, the stored spectra of sample solutions containing 3 µg/ml ALO & 10.8 µg/ml PIO were divided by the absorption spectra of 0.5 µg/ml ALO and 14.4 µg/ml of PIO, respectively, where the obtained ratio spectra were recorded. Difference in amplitude at the selected wavelength were noted and concentration was calculated with the help of regression equations obtained from standard calibration curve. Whereas, in the DR\(^1\), the previously stored ratio spectra of sample solutions were converted to first derivative spectra and amplitude obtained at the selected wavelength were noted and concentration was calculated with the help of regression equations obtained from standard calibration curve. In AUC, the stored zero order absorption spectra of sample solution showed maximum absorption at wavelength at 276 nm and 267.8 nm for ALO & PIO respectively. Therefore, the range 276 ± 10 nm for ALO and 267.8 ± 10 nm for PIO was selected. Then the concentration of sample solution was determined by using the following equation [25].

\[
C_{\text{ALO}} = \frac{\text{AUC}_{(257.8-277.8)} \cdot X_{\text{D}}^{A(266-286)} - \text{AUC}_{(266-286)} \cdot X_{\text{D}}^{A(257.8-277.8)} \cdot X_{\text{A}}^{A(266-286)} - X_{\text{D}}^{A(257.8-277.8)}}{X_{\text{D}}^{A(266-286)} \cdot X_{\text{A}}^{A(257.8-277.8)}}
\]

\[
C_{\text{PIO}} = \frac{\text{AUC}_{(266-286)} \cdot X_{\text{D}}^{A(257.8-277.8)} - \text{AUC}_{(257.8-277.8)} \cdot X_{\text{D}}^{A(266-286)} \cdot X_{\text{A}}^{A(266-286)} - X_{\text{D}}^{A(257.8-277.8)}}{X_{\text{D}}^{A(266-286)} \cdot X_{\text{A}}^{A(257.8-277.8)}}
\]

Where, \(C_{\text{ALO}}\) and \(C_{\text{PIO}}\) are the concentration of ALO and PIO, respectively.

AUC \((257.8-277.8)\) and AUC \((266-286)\) are area under curve of solution at wavelength range between 257.8-277.8 nm and 266-286 nm. \(X_{\text{D}}^{A(257.8-277.8)}\), \(X_{\text{D}}^{A(266-286)}\), \(X_{\text{A}}^{A(257.8-277.8)}\) and \(X_{\text{A}}^{A(266-286)}\) are absorbivities of ALO and PIO at respective wavelength.

2.5 Validation of Spectroscopic Methods

The developed methods were validated in accordance with “International Conference on Harmonization” guidelines [26-28].

2.5.1 Specificity

To check the interference between tablet excipients used in the formulation and drug substance, specificity study was carried out. All the tablet excipients (as per marketed formulation) were mixed in proportion and diluted using methanol and filtered using whatman filter paper no 41. All the solutions (Placebo and standard) were scanned in the UV region and compared to assess the interference among excipients and drugs.

2.5.2 Linearity and range

Linearity and range of all the three methods were checked by analyzing all the standard solutions separately, containing ALO (0.5, 1, 2, 3, 4, 5 µg/ml) and PIO (1.8, 3.6, 7.2, 10.8, 14.4, 18 µg/ml) in methanol and difference in amplitude at 288 nm and 291 nm for ALO, whereas, 236 and 245 nm for PIO were noted for RDSM; 290 and 276.8 nm for DR\(^1\). For AUC, area was noted at 266-286 nm for ALO and 257.8-277.8 nm for PIO. Calibration graphs were constructed using amplitude versus concentration in RD and DR\(^1\); Area versus concentration in AUC. Regression analysis was performed by least squares method to determine the values of slope, intercept and correlation coefficient.

2.5.3 Precision

Precision of the methods were evaluated by performing repeatability (intra-day) and intermediate (inter-day) precision which was expressed in terms of % RSD (Coefficient of variation) of the obtained results. Intra and inter-day precision studies were performed at two dissimilar concentration levels (ALO: 2 & 4 µg/ml; PIO: 7.2 & 14.4 µg/ml) for both the drugs in computer. The wavelengths selected should be such that at each wavelength the absorptivity difference between the two components should be as large as possible. Hence, the \(\lambda_{\text{max}}\) of both drugs was selected for the proposed method. ALO shows maximum absorption at wavelength 276 nm whereas PIO shows maximum absorption at wavelength 267.8 nm. The range 276 ± 10 nm for ALO and 267.8 ± 10 nm for PIO respectively. Therefore, the range 276 ± 10 nm for ALO and 267.8 ± 10 nm for PIO was selected for the AUC. Calibration curve was plotted for ALO at 266-286 nm and 257.8-277.8 nm for PIO.
triplicate on the same day and on three different days, respectively within the linearity range and % RSD of response was calculated. Repeatability of measurement (Instrument precision) for all the methods was checked by analyzing one concentration (ALO: 2 µg/ml; PIO: 7.2 µg/ml) six times for both the drugs and %RSD of the response was calculated.

### 2.5.4 Accuracy
In order to ensure the suitability and reliability of the proposed methods, recovery studies were performed by standard addition method. Known amount of standard drug solutions of ALO and PIO were added to pre-analyzed sample solutions containing ALO (1, 2 and 3 µg/ml) and PIO (3.6, 7.2 and 10.8µg/ml) at three different levels, i.e. 50, 100 and 150%. Finally, % recovery of drugs and RSD (%) was calculated. The outcome of accuracy studies were assessed based on the percentage of standard ALO and PIO recovered from the formulation by applying following formula [27-28]:

\[
\% \text{ Recovery} = \frac{\text{Amount of drug found after addition of standard drug} - \text{Amount of drug found before addition of standard drug}}{\text{Amount of standard drug added}} \times 100
\]

### 2.5.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)
Sensitivity of the proposed methods were determined in terms of LOD and LOQ. The limit of detection and limit of quantification of ALO and PIO were calculated applying following equation [26] as per ICH guidelines.

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S}
\]
\[
\text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where \( \sigma \) = The standard deviation of the response, \( S \) = The slope of the calibration curve.

### 2.6 Stability of the Solution
Stability of the solutions were checked by observing any changes in terms of absorbance and spectral pattern was compared to freshly prepared solutions by keeping the solutions at room temperature and analyzing at a frequent interval.

### 3. RESULTS AND DISCUSSION
The chosen wavelength for RDSM were 291 nm and 288 nm corresponding to a maximum and minimum, respectively for the determination of ALO and 236 nm and 245 nm corresponding to a maximum and minimum, respectively for the determination of PIO. Then the difference in amplitude was calculated at the selected wavelength for both ALO and PIO. The concentration of PIO was computed by using its corresponding equation of linear regression equation produced by plotting the difference in amplitude values at 245 nm and 236 nm (\( \Delta P_{245-236} \)) of the ratio spectra shown in Fig. 3A versus its corresponding concentrations. The concentration of ALO was computed using the corresponding linear regression equation produced by plotting the difference in the amplitude values at 291 nm and 288 nm (\( \Delta P_{291-288} \)) of the ratio spectra shown in Fig. 3B versus its corresponding concentrations.

In this method ALO was successfully determined by dividing the spectrum of mixed standard solutions taking 14.4 µg/ml of PIO as divisor. The obtained ratio spectra were converted into its first order spectra using 1 as scaling factor and 4 as \( \Delta \lambda \). The amount of ALO was determined by measuring the first derivative signal at 290 nm. For PIO a similar procedure was followed using 1 as scaling factor and 2 as \( \Delta \lambda \). Then the amount of PIO was determined by measuring the first derivative signal at 276.8 nm. Amplitudes obtained from the first derivative signal were plotted against corresponding concentration and regression equations were computed. Linear relation was established for ALO and PIO in the concentration range of 0.5-5 µg/ml for ALO and 1.8-18 µg/ml for PIO, respectively. Overlain first derivative of ratio spectra of ALO and PIO are shown in Fig. 4A and 4B.

In AUC, the absorption spectra of the prepared solutions (ALO: 0.5-5 µg/ml; PIO: 1.8-18 µg/ml) were scanned (200–400 nm) and stored in the computer. The wavelengths selected should be such that at each wavelength the absorptivity difference between the two components should be as large as possible. Hence, the \( \lambda_{\text{max}} \) of both drugs was selected for the proposed method. ALO shows maximum absorption at wavelength 276 nm whereas PIO shows maximum absorption at wavelength 267.8 nm. The range 276 ± 10 nm for ALO and 267.8 ± 10 nm for PIO was selected for the AUC. Calibration curve was plotted for ALO at 266-286 nm and 257.8-277.8 nm for PIO. Linear relation was established for ALO and PIO in the concentration range of 0.5-5 µg/ml for ALO and 1.8-18 µg/ml for PIO, respectively. Spectra of ALO and PIO for AUC are shown in Fig. 5A and 5B.
Fig. 3. Overlain ratio spectra of mixture taking 0.5 µg/ml ALO as divisor (A); Overlain ratio spectra of mixture taking 14.4 µg/ml PIO as divisor (B)

Fig. 4. Overlain ratio first derivative spectra of ALO (A); Overlain ratio first derivative spectra of PIO (B)
Fig. 5. UV Spectra of ALO (4 µg/ml), (A) and PIO (14.4 µg/ml), (B) for AUC

Calibration graphs were constructed using amplitude versus concentration in RDSM and DR; Area versus concentration in AUC. Regression analysis was performed by least squares method to determine the values of slope, intercept and correlation coefficient. Regression analysis was performed by applying least square method for calculating values of slope, intercept and correlation coefficient for ALO and PIO at their relative wavelengths. Outcome of precision studies were evaluated in terms of % RSD, follows ICH guideline acceptable limits (<2), which shows good repeatability, low intra and inter-day variability, indicating an excellent precision of the developed methods (Table 1). The outcome of recovery studies ranged from 97-101% for both the drug suggests suitability of the proposed methods (Table 1). Percentage recovery indicates that there was no interference from tablet excipients. Moreover, low LOD and LOQ values prove the sensitivity of the proposed methods (Table 1). Solution stability was checked at room temperature and it was found to be stable up to two days. The projected methods were successfully applied for the quantitative determination of ALO and PIO in laboratory prepared (inhouse) tablet formulation containing 12.5 mg of ALO and 45 mg PIO. Sample solutions were analyzed six times and experimental values were found to be between 98 and 101 % for both the drugs and hence the developed methods can be used for the simultaneous determination of both the drugs in combined tablet formulation (Table 2).
Table 1. Summary of linear regression and method validation data for the proposed methods

| Parameters                      | RDSM | DR1 | AUC  |
|---------------------------------|------|-----|------|
|                                 | ALO  | PIO | ALO  | PIO  | ALO  | PIO  |
| Wavelengths (nm)                | 288-291 | 236-245 | 290 | 276.8 | 266-286 | 257.8-277.8 |
| Linearity range (µg/ml)         | ALO: 0.5-5; PIO: 1.8-18 |  |  |  |  |  |
| Correlation coefficient         | 0.997 | 0.998 | 0.998 | 0.999 | 0.999 | 0.999 |
| Regression equation:            | y = 10.4x + 10.142 | y = 0.2892x - 0.562 | y = 6.1456x + 6.2363 | y = 0.069x - 0.0124 | y = 0.0847x + 0.0105 | y = 0.0943x + 0.2473 |
| LOD (µg/ml)                     | 0.0624 | 0.1486 | 0.0650 | 0.5112 | 0.0686 | 0.3974 |
| LOQ (µg/ml)                     | 0.1892 | 0.4503 | 0.1968 | 1.5489 | 0.2079 | 1.2044 |
| Specificity                     | No interferences |  |  |  |  |  |
| Precision (% RSD)               | 1.4615 | 1.4074 | 1.1711 | 0.4334 | 1.3555 | 1.0305 |
| Repeatability of measurement    | 0.7314 | 1.1302 | 0.8156 | 0.8357 | 1.3624 | 1.4653 |
| Intra-day (n=3)*                | 0.5672 | 0.8400 | 1.1978 | 1.3667 | 1.2678 | 1.4276 |
| Inter-day (n=3)*                | 1.4074 | 1.0518 | 0.5303 | 0.8218 | 0.7808 | 1.0392 |
| % Recovery ± SD                | 99.53±1.40 | 98.91±1.04 | 98.84±0.52 | 99.00±0.81 | 99.42±0.78 | 98.75 ±1.03 |
| % RSD                          | 99.04±1.03 | 99.36±0.86 | 99.46 ± 0.76 | 1.04 | 0.87 | 0.76 |

*n = number of determinations, % RSD (Percentage relative standard deviation)

Table 2. Results of formulation analysis using different methods

| Drugs | Labeled Amount (mg/tab) | Amount Found (mg/tab) | Amount Found (%)* |
|-------|-------------------------|-----------------------|-------------------|
|       | RDSM | DR1 | AUC  | RDSM | DR1 | AUC  | RSD (%) |
| ALO   | 12.5 | 12.38 | 12.40 | 12.41 | 99.04±1.03 | 99.36±0.86 | 99.46 ± 0.76 |
| PIO   | 45   | 44.38 | 44.34 | 44.40 | 98.61±0.98 | 98.69±1.13 | 98.66 ± 1.46 |

*RSD (%) | 1.04 | 0.87 | 0.76 |

*Mean ± SD (n = 6), SD (Standard deviation), % RSD (Percentage relative standard deviation)
4. CONCLUSION

Three different methods namely RDSM, DR$^3$ and AUC were developed for simultaneous estimation of ALO & PIO in combined tablet dosage form. Developed methods were validated according to ICH guidelines. Projected methods were found to be simple, sensitive, precise, accurate and cost effective. Moreover, all the developed UV-spectrophotometric methods require little sample preparation procedure and have wide concentration range with high sensitivity. Therefore, all the developed methods can be used successfully for routine quality control analysis of ALO and PIO in combined tablet dosage form.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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