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Original Article

Ultra HPLC method development and validation for the determination of meclizine in pharmaceutical formulation

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ABSTRACT

For meclizine, a straightforward, quick, and precise high-performance liquid chromatographic (HPLC) method is created, refined, and verified. Linearity, accuracy, and precision were the three criteria used to validate the method. In order to overcome the limitations of the UV approach for determining meclizine concentration, a novel technique called ultra-high-performance liquid chromatography (HPLC) was employed to measure the amount of meclizine in pharmaceutical samples. As a drug it used as antiemetic and for nausea which make it vital for use in different formulations. To assess the amount of meclizine in formulation a quick, easy and reproducible UHPLC method was used. Detection in the device was achieved using UV–A visible detector at a wavelength of 232 nm. A combination of acetonitrile and water (60:30) with 0.7 grammes of monobasic sodium phosphate per 100 mL was optimized to make up the mobile phase. Phosphoric acid was used to bring the pH down to 4. In isocratic mode, the analysis was performed with a 1.5 ml/min mobile phase flow rate. When meclizine was analyzed using UHPLC, it eluted with a distinctively sharp peak with a retention time of 7.6 minutes. The LLOQ, which is equivalent to 1.1 ±0.6 µg/mL, is the lowest concentration. A meclizine limit of detection (LOD) of 0.3 ± 0.5 µg/mL was found. For further research, the approach proved accurate and simple to set up.

Keywords: Meclizine, UHPLC, Calibration curve, Validation, Verification

Introduction

Meclizine is a white to slightly yellowish crystalline powder with a chemical structure of 1-[(4-chlorophenyl) (phenyl) methyl]-4-[(3-methylphenyl) methyl] piperazine. It is insoluble in water, alcohol, chloroform, and dilute acids, with a melting point of approximately 214°C [1]. It is well absorbed from the gastrointestinal tract and has an onset of action within 1 hour, lasting between 8 and 24 hours [2]. However, meclizine shows low oral bioavailability (22–32%) [3]. Meclizine is widely distributed throughout the body, including the central nervous system, and is highly protein-bound. It is primarily excreted through urine with an elimination half-life ranging from 6 to 12 hours [4]. Meclizine treats vertigo and motion sickness-related nausea, vomiting, and dizziness [5]. A popular analytical technique for separating, identifying, and measuring the concentration of each component in a mixture is ultrahigh-performance liquid chromatography (UHPLC). For both quantitative and qualitative examination of pharmaceutical items, it has been determined to be the most accurate analytical technique [6, 7]. The study's objective was to establish an HPLC analytical approach that would be simple, rapid, and accurate for detecting the quantity of meclizine in different formulations. Developing an accurate analytical method is an essential stage in the creation of a novel recipe. The method was verified in compliance with the International Council for Harmonization (ICH) of Technical Requirements for Pharmaceuticals for Human Use guidelines by evaluating its specificity, linearity,

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precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and robustness [8].

Materials and Methods

Meclizine (purity >99%) was obtained from Hangzhou Hyper Chemicals Limited, Zhejiang (China). Monobasic sodium phosphate, Acetonitrile, and water HPLC grade were purchased from Alpha Chemika (India). All other solvents and chemicals were of analytical grade.

Analysis of meclizine via UHPLC

Chromatographic procedures

Acetonitrile and water in a particular ratio (HPLC-grade) solvents, along with an appropriate quantity of monobasic sodium phosphate in each 100 mL, served as the mobile phase for the analysis. Phosphoric acid was employed to bring the pH down to 4. The UHPLC used was Ultra HPLC- PDA (LC-2060C 3D) (Shimadzu®, Japan). The results were analyzed using LabSolutions® LC workstation PC software (Ver.5). Shim-pack® XR-ODS II (150 mm × 3.0 mm I.D., 2.2 µm particle diameter) with detection at 200-400 nm wavelength was used. The mobile phase was always clarified by passing it through a nylon micro filter paper with a pore size equal to 70 nm, degassing it with a bath sonicator, and then pumping it at a predetermined optimized flow rate in isocratic mode. The temperature of the oven was 40 ±1 °C, the cooler was 5 ±1 °C, and the injection volume was 10 µL. A 25-minute run time was optimized for the procedure. At various wavelengths between 200 and 400 nm, the photodiode array detector assessed the height and peak area of a particular peak of the sample or analyte. The wavelength was set at 254 nm (channel 1). The high-intensity region, which had detected UV absorption for the separated sample, was chosen as the extracted chromatogram. The chromatogram retention time was optimized, and meclizine was detected from the eluted high-resolution peak [9, 10].

Preparation of meclizine standard solutions

In the isocratic mode, a stock solution of meclizine (1 mg/mL) was made in the mobile phase (acetonitrile: water) with an ideal volume ratio. Standard meclizine solutions were made by serially diluting the stock solution with the mobile phase, yielding concentrations of pure meclizine that ranged from 5, 10, 20, 40, 80, and 160µg/mL. The calibration curve was constructed for the standard solutions of meclizine after UHPLC analysis. Each measurement was done in triplicate [11].

Chromatographic conditions optimization

Optimization of the chromatographic conditions was performed by optimization of the following parameters [12]:

Mobile phase ratio

Meclizine in samples was analyzed by optimizing the mobile phase ratio in isocratic mode. Acetonitrile: Water (%, v/v) ratios of 25:75, 50:50, 75:25, 40:60, and 60:40 was among the many ratios that were examined. It was found that the mobile phase ratio that resulted in a greater recovery and better resolution was ideal for the analysis.

Mobile phase flow rate

After applying different flow rates (1,1.5, and 2 mL/min), respectively, for optimization reasons, the mobile phase flow rate was changed in the isocratic mode for the analysis of the compound under study.

Detector's wavelength

The wavelength of the detector was measured between 200 and 400 nm in order to identify meclizine. To analyze the substances under study, the wavelength that produced the best sensitivity and resolution was selected.

Verification of the method of analysis

The following standards were used to validate the suggested analytical technique:

Linearity

The calibration curve created at multiple concentration levels (5-160 µg/mL of meclizine) demonstrated the linearity of the suggested approach. Using a linear least squares regression analysis, a calibration curve was created for meclizine in the mobile phase by charting peak regions versus corresponding concentrations [13].

Selectivity and specificity

The term "selectivity" describes a method's capacity to distinguish one analyte from another in a complicated mixture without interference from other substances. However, the highest level of selectivity, or 100% selectivity (or 0% interference), is known as specificity. Verifying the full separation and resolution of the necessary peak region of meclizine in the mobile phase allowed researchers to examine the specificity and selectivity of the analytical technique [14].

Lower limits of detection and quantification

A 10 µL sample was injected utilizing the dilution technique using a signal-to-noise (S/N) methodology to determine detection and quantification limits. The lower limit of quantification was defined as a minimum concentration with a signal-to-noise ratio of at least ten (S/N≈10), as indicated in Eqs. 1 and 2, respectively, and the lower limit of detection as a minimum concentration with a signal-to-noise ratio of at least three (S/N≈3.3) [15].

$$\text{LLOD} = 3.3 \times \frac{\text{SD}}{\text{S}} \quad (1)$$

$$\text{LLOQ} = 10 \times \frac{\text{SD}}{\text{S}} \quad (2)$$

Where SD is the standard deviation of the measured concentrations and S = Mean of the slope of the calibration curve.

Accuracy

The proposed method's accuracy was calculated as a percentage of recovery. Standard solutions of meclizine were prepared in the mobile phase. The sample readings were done in triplicate [16]. The percentage recovery for meclizine was calculated using Eq. 3:

$$\%\ \text{Recovery} = \frac{[\text{A}]}{[\text{B}]} \times 100\% \quad (3)$$

Where [A] is the net peak area of the meclizine in an unknown sample, [B] is the peak area of the meclizine in the standard solution.

Precision

The precision of the method was assessed in terms of intermediate precision (intra-day and inter-day reproducibility) and repeatability (injection and analysis). To evaluate injection repeatability, five independent samples of reference meclizine solutions containing 5, 10, 15, 20, and 25 µg/mL were injected three times each into the UHPLC system [17]. By calculating the percent relative standard deviation (%RSD) from the collected data and assessing the repeatability of the relative peak area, which was represented as mean ± SD, the accuracy of the suggested method was ascertained. To obtain the intermediate precision (intra-day and inter-day repeatability), standard solutions in the mobile phase at the five different concentration levels were prepared and analyzed three times daily for three consecutive days. Mean ±SD and %RSD were used to indicate the relative peak areas based on the data gathered [18, 19].

System suitability parameters

A system suitability measurement is used to check the sensitivity, resolution, and reproducibility of the chromatographic system and see if they are well enough for the analysis to be done. The parameters mainly used in system suitability are the resolution (R), repeatability, standard deviation of peak response, retention time, column efficiency, the tailing factor (coefficient that shows the degree of peak symmetry, height equivalent to the theoretical plate factor (HETP) which is affected by temperature, column length and size of particles inside the column, number of theoretical plates and column efficiency [20, 21].

Stability

The stability studies of meclizine standard solutions were carried out at (40 ±1 °C) and a refrigerator (2–8 °C) to determine if the samples were stable enough to give a high-resolution peak in UHPLC or decomposed as an effect of temperature, which might affect on the determination of meclizine in the stored samples [22, 23].

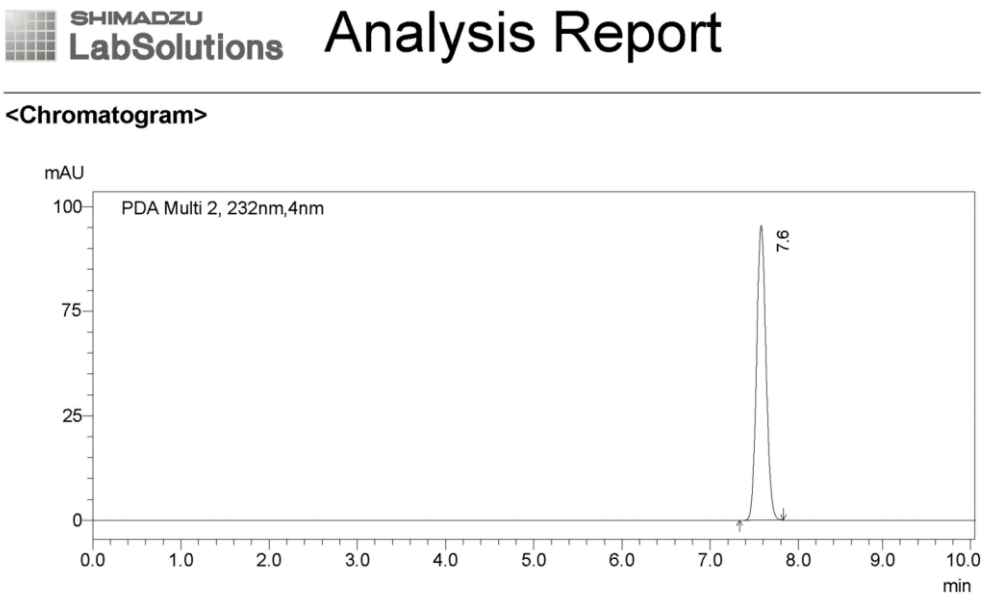

Figure 1. Chromatogram of meclizine using mobile phase (Acetonitrile: Water 60:40)

Results and Discussion

The analysis results revealed that a 1.5 mL/min flow rate was effective for better separation. The chromatogram showed the retention time was optimized at (7.6 ±0.05 min) as shown in **Figure 1**, in which the photodiode array detector showed an absorption peak at 232 nm, gradually decreasing in intensity as it moved away in the far UV region as shown in the contour **Figure 2**.

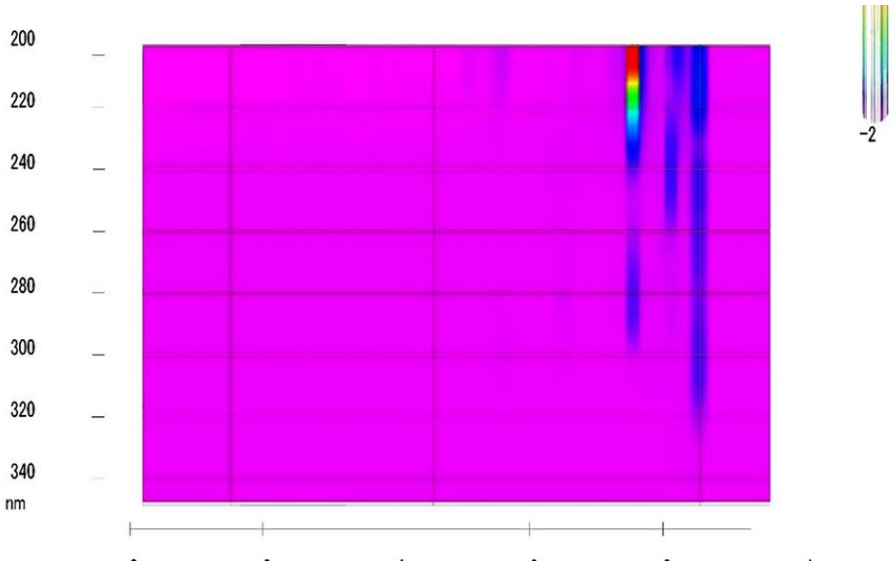

Figure 2. UV region as shown in the contour

Verification of the analysis method

Linearity

The calibration curve of the standard solutions at various meclizine concentration levels was used to determine the method's linearity. The peak area yielded a linear correlation over a 5-160 µg/mL concentration range. In addition, the line's calibration curve and the respective correlation coefficient (R2) are all shown in **Figure 3**.

Specificity/Selectivity

Chromatograms of standard solutions of meclizine at concentrations ranging from 5-160 µg/mL confirmed that meclizine was well resolved and completely separated at a retention time of (7.6 ±0.05 min) as shown in **Figure 4** [22, 24].

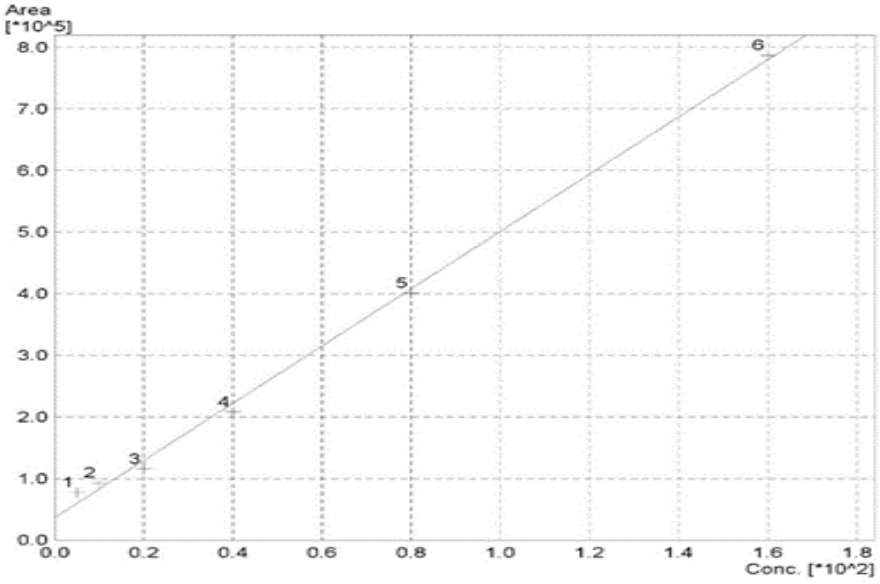

Figure 3. Calibration curve of meclizine in the mobile phase (Acet: Water), (60:40 v/v). (n=3)

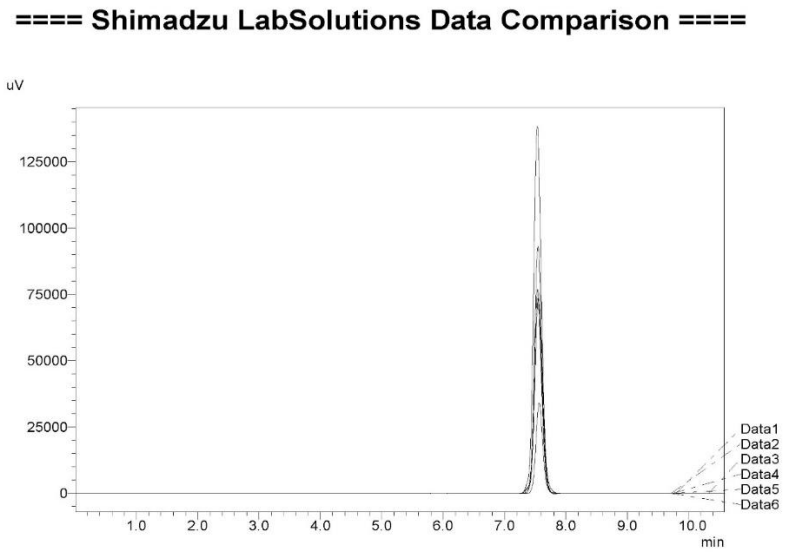

Figure 4. Stacked chromatograms of the measured standard conc. of meclizine. (n=3)

The lower limit of detection and quantification

Six concentrations, including the lower limit of quantitation (LLOQ), were used to confirm the linearity of the analytical technique. 1.8 ±0.1 µg/mL is the lowest concentration or LLOQ. The detection limit (LOD) for meclizine was 0.6 ± 0.05 µg/mL. These results confirmed the great sensitivity of the suggested analytical approach [25, 26].

Accuracy

The mobile phase was spiked with a suitable amount of the meclizine stock solution to provide 80, 100, and 120% of the theoretical concentration. According to **Table 1**, the average recovery of the spiked samples was between 98.24 ±0.15% and 100.03 ±0.48%. The chromatograms (A, B, and C) of meclizine are shown in **(Figures 5-7)**, respectively.

Table 1. Accuracy (% Recovery) of meclizine. (n=3)

(%) of Meclizine	Area	Area recovered	%Recovery ± SD
80	67809	66616	98.24 ±0.15%
	65015	64896	99.81 ±0.09%
	60728	60747	100.03 ±0.48%
100	72968	72039	98.72 ±0.31%
	71820	70591	98.28 ±0.29%
	72051	71289	98.94 ±0.17%
120	78510	78069	99.43 ±0.26%
	79675	79522	99.80 ±0.62%
	83201	82759	99.468 ±0.45%

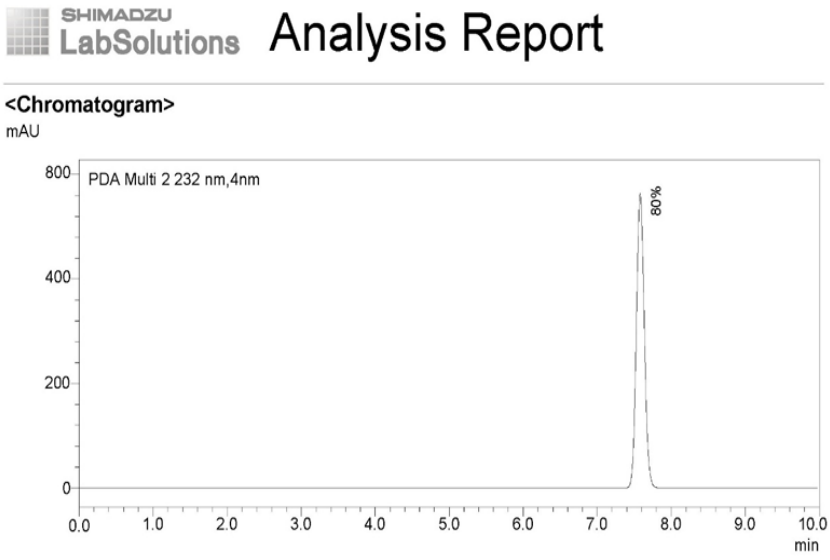

Figure 5. Chromatogram of meclizine at 80% amount. (n=3)

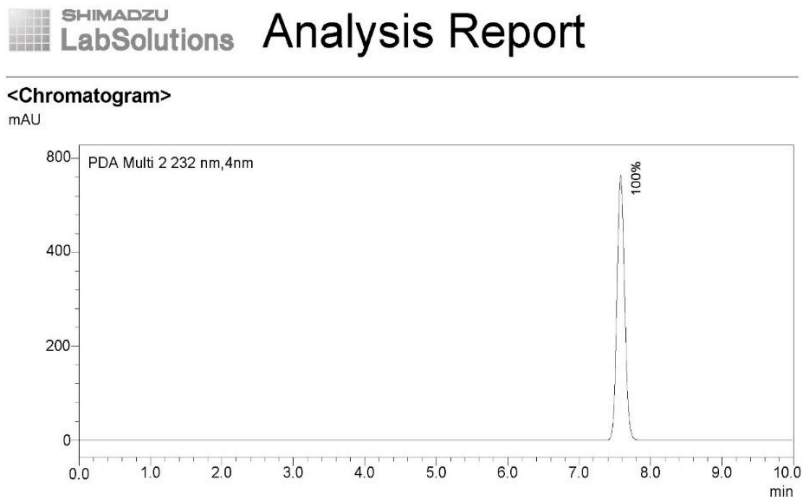

Figure 6. Chromatogram of meclizine at 100% amount. (n=3)

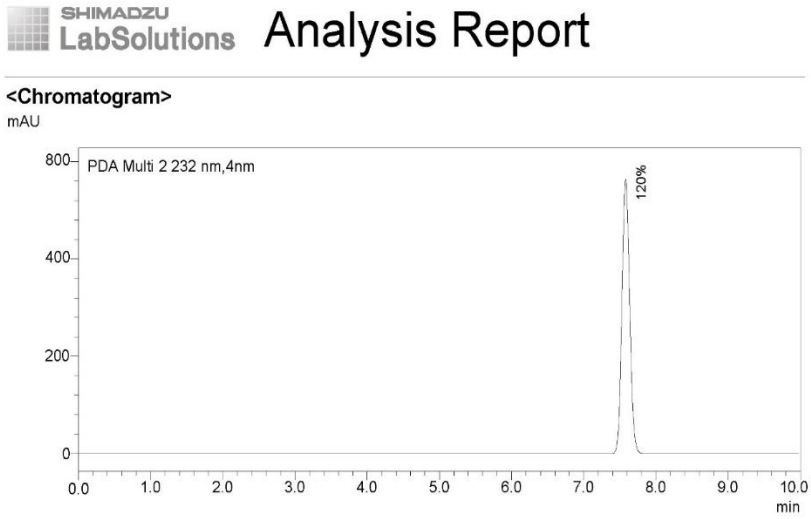

Figure 7. Chromatogram of meclizine at 120% amount. (n=3)

Precision

Intra-day precision was performed by analyzing standard solutions within the calibration range three times on the same day. Inter-day precision was performed by analyzing meclizine solutions within the calibration range on three days, as shown in **Table 2**. However, intra-day and inter-day relative standard deviation (% RSD) values for standard solutions for meclizine were less than 2%.

Table 2. Precision of Repeatability and Reproducibility

Mean relative peak area ±SD, %RSD			
Meclizine Conc. (µg/mL)	**Meclizine Conc. (µg/mL)**	**Meclizine Conc. (µg/mL)**	**Meclizine Conc. (µg/mL)**
5	5	5	5
10	10	10	10
15	15	15	15
20	20	20	20
25	25	25	25

System suitability parameters

The parameters mainly used in system suitability are the tailing factor, the theoretical plate number, and retention time. Results are shown in **Table 3** [27].

Stability

Results obtained from the stability study of the standard solution (5 µg/mL) of meclizine showed that it was stable for 7 h at (40 ±1°C), and for 5 days in a refrigerator (4 ±1 °C); after that, it started to degrade.

Table 3. Acceptance Criteria of System Suitability Parameters

(%) of meclizine	Area	Area recovered
Parameter	Limit	Experimental
Tailing factor	<2	1.075±0.9
Theoretical Plates (USP)	>2000	20611±4

Conclusion

In accordance with ICH recommendations, an effective high-performance liquid chromatography (UHPLC) technique was created, refined, and verified to detect and quantify meclizine in samples. Peak quality, analysis time, and resolution were assessed and adjusted. It was discovered that the approach was linear, sensitive, specific, accurate, and precise. Controlling the wavelength and mobile phase flow rate was crucial since they seemed to have a substantial (0.05>P) impact on robustness. Therefore, laboratories might apply the suggested approach to measure the amount of meclizine. It was considered a novel method developed to analyze the drug using advanced devices for analysis.

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Conflict of interest: None

Financial support: None

Ethics statement: None

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