



Research Article

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A SIMPLE RP-HPLC METHOD FOR SIMULTANEOUS ANALYSIS OF PARACETAMOL, NIMESULIDE AND TIZANIDINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A novel, simple and economic reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the estimation of Paracetamol, nimesulide and tizanidine in bulk and tablet dosage forms with greater precision and accuracy. Separation was achieved on C18 column (250X4.6mm, 5 μ m) using gradient mobile phase Sol-A: buffer (weighed accurately 1gm of ammonium acetate in to 1000mL of HPLC grade water) and Sol-B: acetonitrile gradient program (0-6min, sol-A: 80-37; 6-10min- sol-A: 37-80 and 10-15min- sol-A: 80-80), pumped in to the column at flow rate of 1 ml/min and the detection of eluent from the column was carried out using variable wavelength detector at 230nm. The total run time was 15 min and the column was maintained at ambient temperature. The retention times of paracetamol, tizanidine and nimesulide were 3.0min, 5.3 and 8.6min, respectively. The standard curves were linear over the concentration range of 12.5-75 μ g/ml and the % RSD of intraday and inter day precision was found to be good. The method was validated as per ICH guidelines. Validation studies demonstrated that the proposed RP-HPLC method is simple, specific, rapid, reliable and reproducible. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the routine quality control analysis in bulk and tablet dosage forms.

Key words: Paracetamol, Nimesulide, Tizanidine, RP-HPLC and solid dosage forms.

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INTRODUCTION

Paracetamol (acetaminophen) is one of the most popular over-the-counter (OTC) analgesic and antipyretic drugs. Paracetamol⁽¹⁻⁸⁾ is available in different dosage forms: tablet, capsules, drops, elixirs, suspensions and suppositories. Nimesulide, N-(4-Nitro-2-phenoxyphenyl) methane sulfonamide is a non-steroidal anti-inflammatory analgesic drug has a multifactorial mechanism of action that affects the activity of MMPs (metalloprotease) and other biochemical markers of joint destruction, reduces the release of ROS (reactive oxygen species) and other toxic substances from neutrophils and reduces the production of pro inflammatory cytokines. Nimesulide⁽⁹⁻¹³⁾ has a rapid onset of the analgesic action. Its approved indications are the treatment of acute pain, the symptomatic treatment of osteoarthritis and primary dysmenorrhea in adolescents and adults above 12 years old. These unique characteristics make nimesulide an appealing therapeutic choice in the treatment of acute pain.

Tizanidine⁽¹⁴⁾ (5-chloro-4-(2-imidazolin-2-ylamino)-2, 1, 3-benzothiadiazole) is a 2- adrenergic agonist and centrally active myotonolytic skeletal muscle relaxant with a chemical structure unrelated to other muscle relaxants. Figure-1 represents the chemical structure of all active ingredients.



Figure-1: Chemical structures of active ingredients

Numerous methods have been reported for the analysis of paracetamol and its combinations in pharmaceuticals or in biological fluids. Paracetamol has been determined in combination with other drugs using titrimetry, voltammetry, fluorimetry, colorimetry, UV-spectrophotometry, quantitative thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and gas chromatography (GC) in pharmaceutical preparations. The main objective of this study is to develop and single, high accurate RP-HPLC method for the analysis of Paracetamol, Tizanidine and Nimesulide in pharmaceutical dosage forms.

MATERIALS AND METHOD

Instruments: A waters HPLC system consisting of alliance 2695, agilent 1200 series HPLC instrument with UV-Visible detector, two systems were operated by empower software. A Novapack C18

250×4.6mm, 5µm column, Mettler Toledo analytical balance was used for this study.

Materials: Pure (not less than 98.5%) standards of all active ingredients, HPLC grade acetonitrile and water; AR grade of ammonium acetate were used.

Mobile phase: Sol-A: weighed accurately 1000mg of ammonium acetate, transferred in to 1000ml of HPLC water and mixed. Filtered the final solution through a 0.4µ membrane filter; Sol-B: HPLC grade acetonitrile.

Diluent: Mixed the HPLC water and acetonitrile in the ratio of 1:1 (v/v) and degassed.

Standard solution: Prepared the standard solution to get each active ingredient equal to 50microgram per mL with diluent.

Test solution: Prepared the all dosage forms to get each active ingredient equal to 50microgram per mL with diluent and analyzed.

Chromatographic conditions

Chromatograph : Waters/ Agilent HPLC system with Empower software.

Mobile phase : Solution-A and solution-B with gradient elution.

Gradient program : (0-6min, sol-A: 80-37; 6-10min- sol-A: 37-80; 10-15min- sol-A: 80-80 ;)

Column : Novapack C18 250×4.6mm, 5µ.

Flow rate : 1.0 mL per min

Detection : 230nm

Injection volume : 10 µl

Retention time : Paracetamol-3.0min, Tizanidine – 5.3min and Nimesulide -8.6min.

Run time : 15 min.

Calculation : All active ingredients were quantified with the following calculation.

$$\frac{\text{Sample area} \times \text{standard concentration} \times \text{Potency of standard}}{\text{Standard area} \times \text{sample concentration} \times 100} \times 100$$

RESULTS AND DISCUSSION

Method Development

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water and buffer solutions in various proportions and at different pH values. Flow rates between 0.5 and 1.5/min were studied. A flow rate of 1.0 ml/min gave an optimal signal to noise ratio with a reasonable separation time using a reversed-phase C18 column. Total time of analysis was less than

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15min. The maximum absorption of paracetamol, tizanidine and nimsulide together as detected at 230nm and this wavelength was chosen for the analysis. The chromatogram at 230nm showed a complete resolution of all peaks. Figure-2 and 3 represents the diluent and standard solution chromatograms.

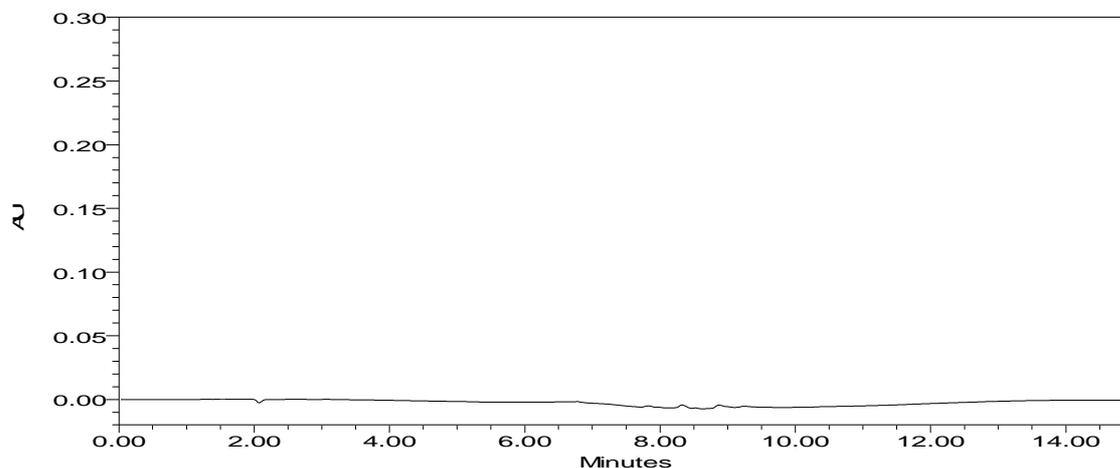


Figure-2: Diluent chromatogram

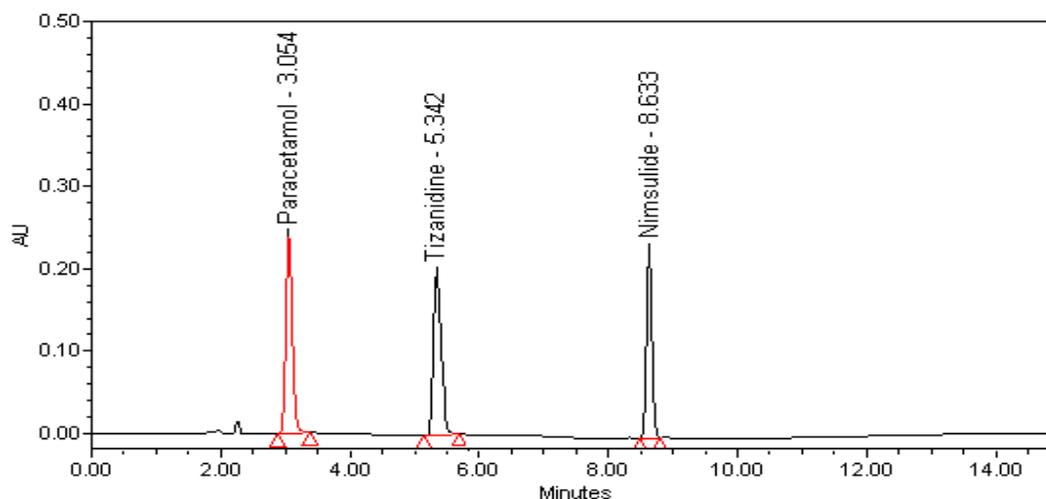


Figure-3: Standard chromatogram

System suitability:

System suitability parameters were established by injecting the freshly prepared standard solution (each active 50microgram per mL/five replicate injections) in to the chromatographic system. The percent relative standard deviation for peak area and retention time results found to be satisfactory. System suitability chromatograms were represented in figure-4 and tabulated the results in table-1 to 4.

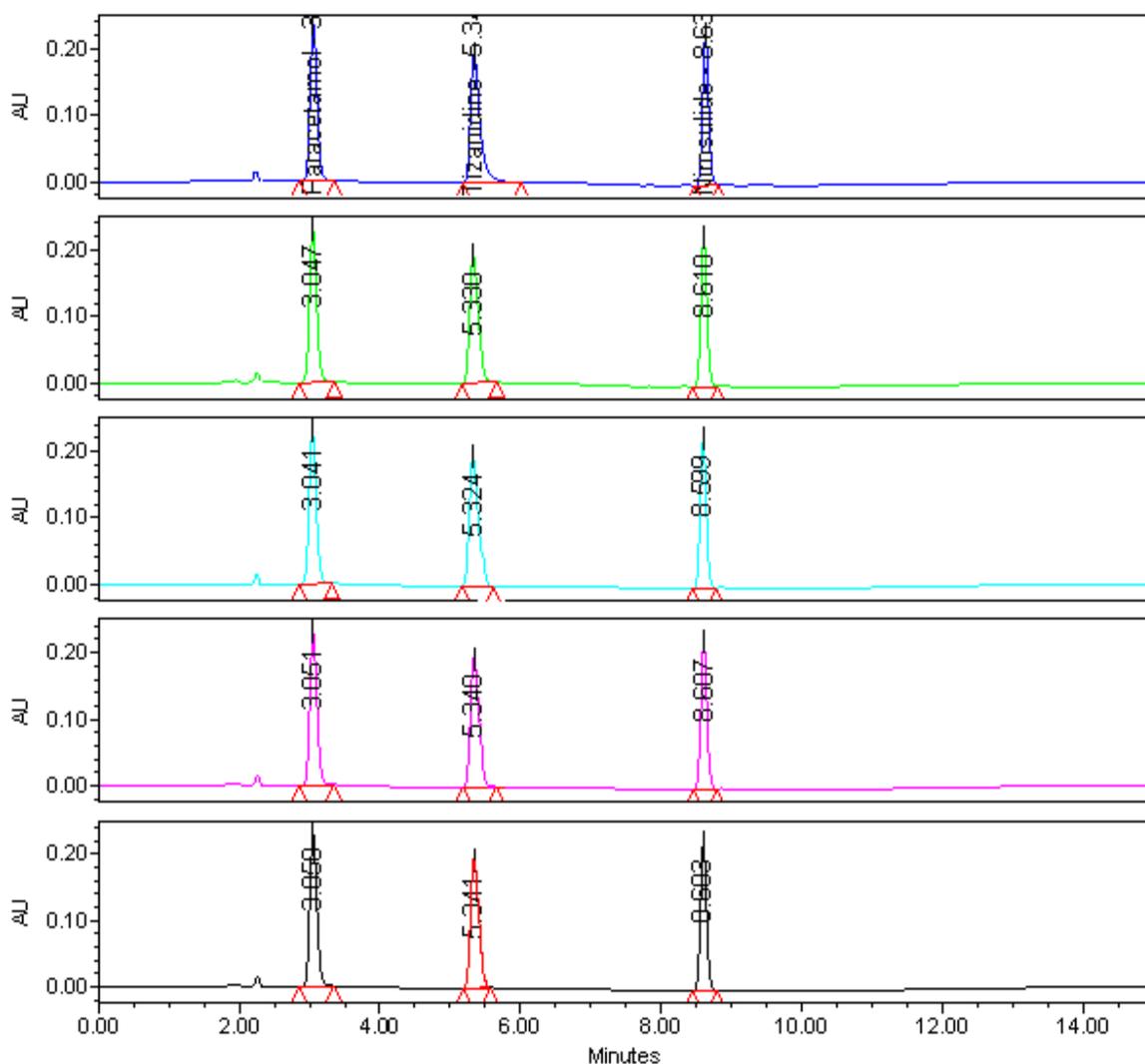


Figure-4: System suitability chromatograms

Table-1: System suitability (Area %RSD)

Active Name	Standard solution Area						%RSD
	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Average	
Paracetamol	1656904	1652164	1649792	1646380	1648019	1650652	0.25
Tizanidine	1884227	1850791	1830840	1808919	1836268	1842209	1.51
Nimesulide	1349448	1354795	1352221	1349856	1350374	1351339	0.16

Table-2: System suitability (Retention time %RSD)

Active Ingredient Name	Standard solution Retention time (min)						
	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Average	%RSD
Paracetamol	3.05	3.04	3.04	3.05	3.05	3.05	0.18
Tizanidine	5.34	5.33	5.32	5.34	5.34	5.33	0.17
Nimesulide	8.63	8.61	8.59	8.61	8.60	8.61	0.17

Table-3: System suitability (USP Tailing factor)

Active Ingredient Name	Standard solution Retention time (min)				
	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5
Paracetamol	1.1	1.1	1.1	1.1	1.1
Tizanidine	1.7	1.6	1.6	1.6	1.6
Nimesulide	1.1	1.1	1.1	1.1	1.1

Table-4: System suitability (Resolution)

Active Ingredient Name	Standard solution Retention time (min)				
	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5
Resolution between Paracetamol and Tizanidine	10.53	10.41	10.45	10.44	10.43
Resolution between Tizanidine and Nimesulide	16.13	15.98	16.11	15.86	15.90

Precision: The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated. From the data obtained, the developed HPLC method was found to be precise.

Linearity: Linearity is determined by calculating the regression line using a mathematical treatment of the linearity results vs analyte concentration (10microgram per mL to 60microgram per mL for each ingredient) of the standard solution. Linearity graph was plotted against peak area and concentration of solution. The correlation coefficient value found to be within the limit 0.999. The linearity plots were represented in figure-5 and chromatograms shown in figure-6 and linearity results tabulated in table-5.

Table-5: Linearity Results

Active Name	Linearity Results						Co-relation Coefficient
	25% (12.5ppm)	50% (25ppm)	75% (37.5ppm)	100% (50ppm)	125% (62.5ppm)	150% (75ppm)	
Paracetamol	300984	755226	1178848	1653147	2102392	2518383	0.9999
Tizanidine	295522	757472	1185127	1696791	2163186	2559251	0.9995
Nimesulide	243925	613779	958384	1330869	1695619	2074231	0.9999

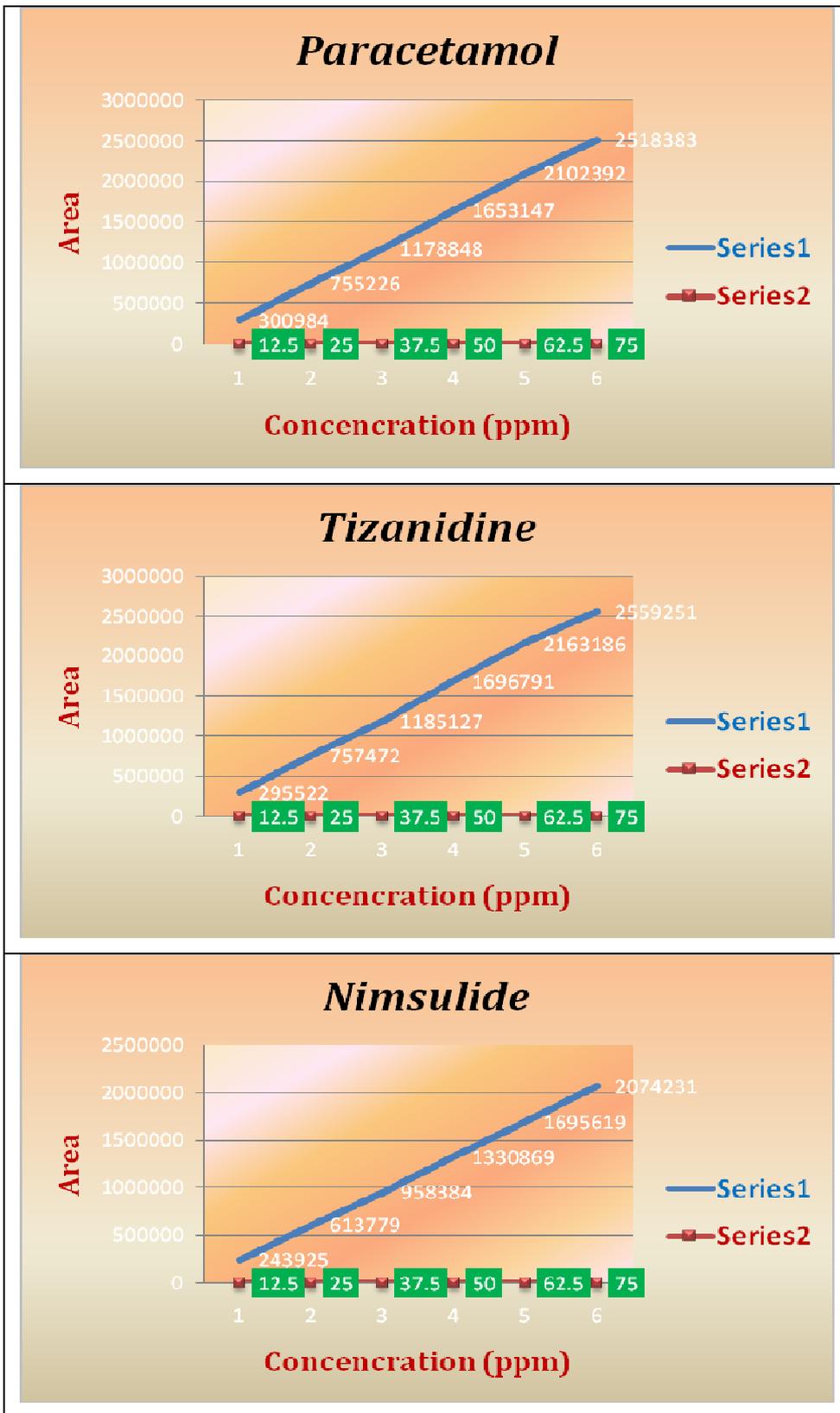


Figure-5: Linearity Graphs

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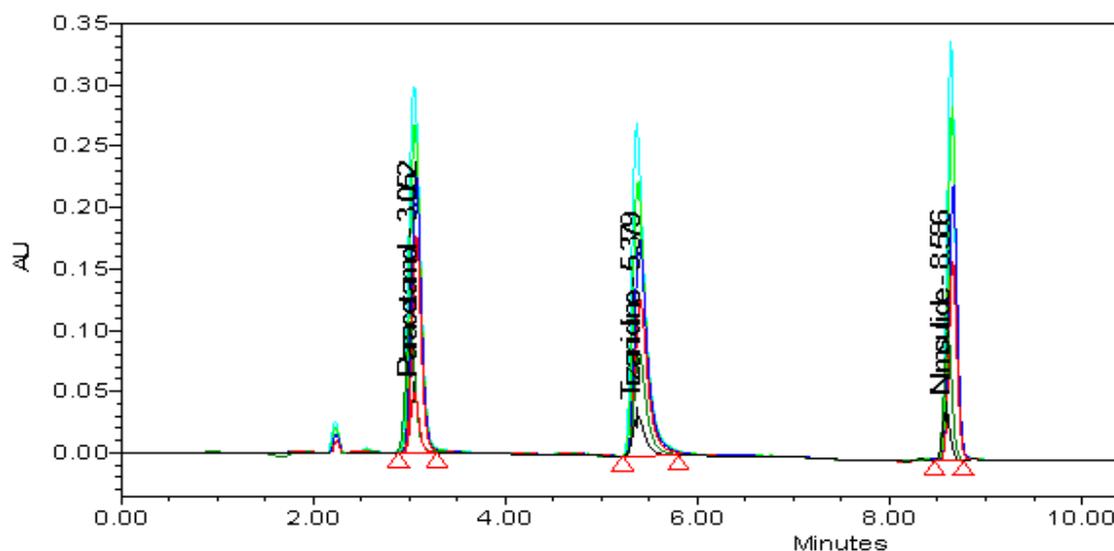


Figure-6: Linearity chromatograms

Recovery: Recovery studies were performed by adding the active ingredient to placebo at different levels from 25% of test concentration to 150% of test concentration. Results were found to be satisfactory and the results found in the range from 99 to 101.11%.

Robustness and Robustness: Each factor selected (except columns from different manufacturers) was changed at three levels (-0.1, 0 and 0.1). One factor at the time was changed to estimate the effect. Thus, replicate injections ($n = 6$) of mixed standard solution at three concentration levels were performed under small changes of three chromatographic parameters (factors). Insignificant differences in peak areas and less variability in retention time were observed and results (system suitability results, assay values) were found to be satisfactory.

CONCLUSION

A gradient RP-HPLC method developed and validated for the simultaneous determination of Paracetamol Tizanidine and Nimesulide in both bulk and tablet dosage form. The validation results reveals that, method have good precision and accuracy, which proves the reliability of the proposed method. The short runtime and low solvent consumption are advantageous for applying equal quality control analysis.

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