METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF FLUPENTIXOL AND ESCITALOPRAM BY USING RP-HPLC

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Abstract

The aim of this study is to create and validate a fast, easy-to-use, affordable, sensitive, and accurate method for measuring Escitalopram and Flupentixol in bulk and pharmaceutical products using Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC). Agilent Eclipse XDB C_{18} column was used with a running phase composed of 0.1% Tri fluoro acetic acid: Acetonitrile (30:70 v/v) at a flow rate of 1.0 ml/min. UV detection was used at a wavelength of 231 nm. Flupentixol and Escitalopram correlation coefficients were found to be 0.999 over a concentration range of 1.25-7.5 µg/ml and 25-150 µg/ml, respectively. Escitalopram and Flupentixol have respective retention times of 3.235 and 3.858 minutes. The run time for separating Flupentixol and Escitalopram peaks was 6 minutes. This method, proposed as a regular analysis and quality control tool for medications that contain these active drugs either individually or in combinatio, was evident to be a suitable one.

Keywords: *HPLC, Flupentixol, Escitalopram, Development, Validation.*

1. Introduction

Flupentixol (INN), also known as **flupenthixol** (former BAN), marketed under brand names such as **Depixol** and **Fluanxol** is a typical antipsychotic[1-2] drug of the thioxanthene class. In addition to single drug preparations, it is also available as flupentixol/melitracen—a combination product containing both melitracen (a tricyclic antidepressant[3-4]) and flupentixol. Flupentixol's main use is as a long-acting injection given once in every two or three weeks to individuals with schizophrenia[5-6] who have poor compliance with medication and suffer frequent relapses of illness, though it is also commonly given as a tablet. There is little formal evidence to support its use for this indication but it has been in use for over fifty years. Flupentixol is also used in low doses as an antidepressant. There is tentative evidence that it reduces the rate of deliberate self-harm, among those who self-harm repeatedly.

Escitalopram, sold under the brand names Cipralex and Lexapro, among others, is an antidepressant of the selective serotonin[7-8] reuptake inhibitor (SSRI) class. Escitalopram is mainly used to treat major depressive disorder or generalized anxiety disorder[9-10]. It is taken by mouth. It is available commercially exclusively as the oxalate salt. Common side effects include trouble sleeping, nausea, sexual problems[11-12], and feeling tired. More serious side effects may include suicide in people

under the age of 25. It is unclear if use during pregnancy or breastfeeding is safe. Escitalopram is the (*S*)-stereoisomer (left-handed version) of citalopram (which exists as a racemate), hence the name *es*citalopram. In other words, escitalopram is a chiral switch of citalopram. Simultaneous determination of Escitalopram and Flupentixol using HPLC was found in the current study.

2. Experimental Study

Solutions and Reagents

The pure Flupentixol and Escitalopram used in this study was provided by Glenmark Pharmaceutical Private Ltd., located in Andheri (E), Mumbai, India (99.8-99.9 percent purity). Other reagents, including acetonitrile, tri fluoro acetic acid, and water, were obtained from Merck (India) Ltd. in Worli, Mumbai, India, and were of HPLC grade.

Collection of instruments

Waters alliance liquid chromatography (model 2695) monitored with empower 2.0 data handling system and a detector of photo diode array (model 2998) was used for this study.

Buffers are chosen

0.1 percent Tri fluoro acetic acid.

Step of mobility

For Standard review, the mobile step was 0.1% Tri fluoro acetic acid buffer in a 30:70 (v/v) Acetonitrile mixture and was degassed beforehand. A mobile phase chosen to produce well-defined peaks with a low tailing factor (2.0) and a plate count of over 2000 was selected.

Prepare the diluent

Mobile phase was used as diluent.

Conditions of Chromatography

For the HPLC experiments, an agilent eclipse XDB column (250 x 4.6 mm, 5 µm) was used. The elution was conducted with isocratic conditions using Acetonitrile: tri fluoro acetic acid (0.1% volume) (70:30 by volume) at a flow rate of 1.0 ml/min. The injection volume was 10 µl, and the run time was 6 minutes, with the column temperature set to room temperature and the absorbance measured at 231 nm (Because maximum absorbance was observed at this wavelength. So, this was selected as wavelength).

Standard Solution Preparation

To dilute 5 mg of Flupentixol and 100 mg of Escitalopram, measure out the drug and transfer it to a 100 mL volumetric flask, then add approximately 70 mL of a diluent and sonicate for 30 minutes to dissolve it, after that add more diluent to the total amount. Further dilute 5 mL to 50 mL with diluents.

Sample Solution Preparation

Measure out 97 mg of sample and transfer it to a 100 mL volumetric flask, then add approximately 70 mL of a diluent and sonicate for 30 minutes to dissolve it, after that add more diluent to the total amount.

Validation Process[13-21]

System Precision

The system's performance has been validated through assessment of device suitability parameters. Limits were found to be met for a variety of parameters, including plate count, tailing, and RSD percentage.

Specificity

Being able to identify and test a given analyte in the presence of other elements required to be combined in the Standard and the standard solution is known as specificity. Blank Standards and those with Flupentixol and Escitalopram will be tested using chromatograms.

Accuracy

Being close to the real meaning of the technique is what defines accuracy. Three concentrations will be used to test the recovery trials. The drug's quantity, percentage of recovery, and standard deviations were calculated after every injection at each level.

Precision

It is the level of agreement between the various test results that determines the precision of the analytical methodology. Researchers examined the effects of sampling a homogeneous population more than once. The current process was evaluated in terms of its ability to provide repeatable, intraday, and inter-day results. It was examined by sampling the materials on the same day and over the course of different days.

Linearity

Linearity is the feature of analytical process which allows for a direct proportion of analytical results in response to a certain concentration of the analyte in the Standard. A total of seven series of standard solutions were selected for the assessment of the linearity spectrum. The calibration curve was drawn by comparing regular solution concentration with peak area. Using the least square method, the slope, intercept, and coefficient of correlation were calculated.

Forced Degradation

The peaks in the chromatogram should agree. ICH guidance Q1 (A) R2 was performed in conjunction with stress degradation experiments. The peaks of degradation should be well distanced and at least 1.0 resolution between peaks. For the largest peaks to go over, a separation must occur. A degradation of around 20 percent has been attained via several various stress conditions like acid, alkali, peroxide, reduction, thermal and photo in what is known as a forced degradation experiment.

Robustness

Robustness refers to a procedure's resistance to small process parameter changes, as well as its reliability in normal operation. An organic solution was introduced into the HPLC system for a robustness analysis, and the chromatographic settings (such as flow rate and mobile-phase organic content) were modified. The separation factor, retention time, and peak asymmetry were determined by evaluating the effects of altered parameters.

3. Results and Discussion

The aim of this study is to establish a single isocratic HPLC method for the simultaneous quantification of Flupentixol and Escitalopram in bulk and pharmaceutical dosage forms that is reliable, precise, and cost effective. According to the UV spectra of these compounds, an appropriate wavelength for simultaneous estimation of two drugs was chosen.

Optimization of the method

The separation was achieved using agilent eclipse XDB column (250mm x 4.6mm, 5µm) and a mobile phase of 0.1% tri fluoro acetic acid : acetonitrile (30:70 v/v) with a flow rate of 1.0 ml/min and UV detection at a wavelength of 231 nm. The entire performance lasted six minutes. Conditions for optimized chromatography are provided in table 1.

System Suitability

To attain results, the following device suitability parameters were established after six consecutive injections of normal solution: theoretical plate number, time, peak area, tailing factor, and resolution. The chromatogram in Figure 1 was the representative of the suitability results detailed in table 2.

Table 1. Method suitability conditions

Parameter	Suitable conditions
Column	Agilent eclipse XDB (250 x 4.6 mm, 5 µ)
Moving Phase	0.1% tri fluoro acetic acid: Acetonitrile (30:70 v/v)

Volume of injection	10 µl
Stream rate	1.0 mL/min
Temperature of column	25°C
Wavelength	231 nm
Time duration	6minutes

Table 2. Results of system suitability

Parameter	Escitalopram	Flupentixol
Number of plates	7587	2659
Tailing	0.99	0.89
Resolution		2.87
Peak elution time	3.235	3.858
% RSD	0.31	0.63

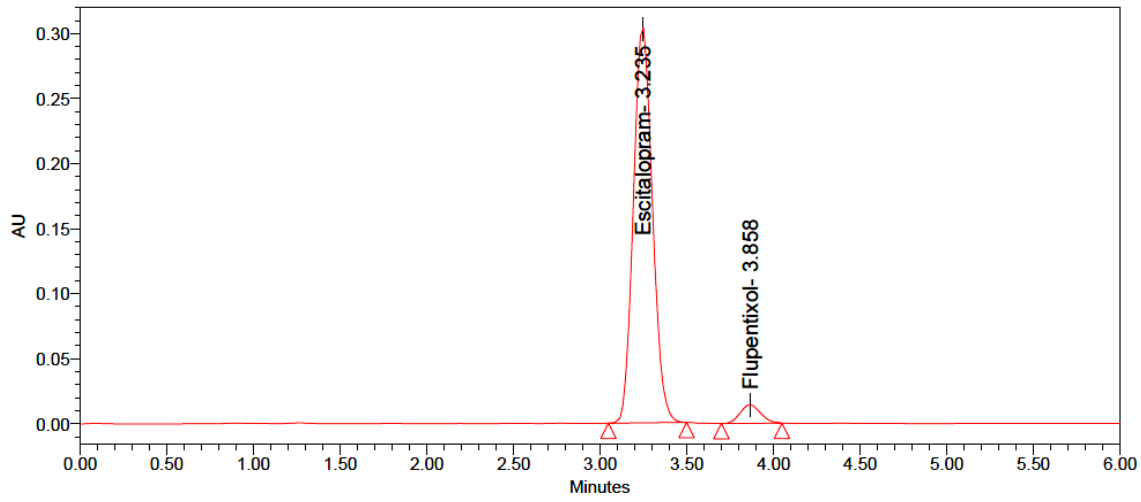

Figure 1. Chromatogram of standard

Specificity

There was no participation from Flupentixol and Escitalopram at the elution time. As seen in Figure 2, the blank chromatogram is present.

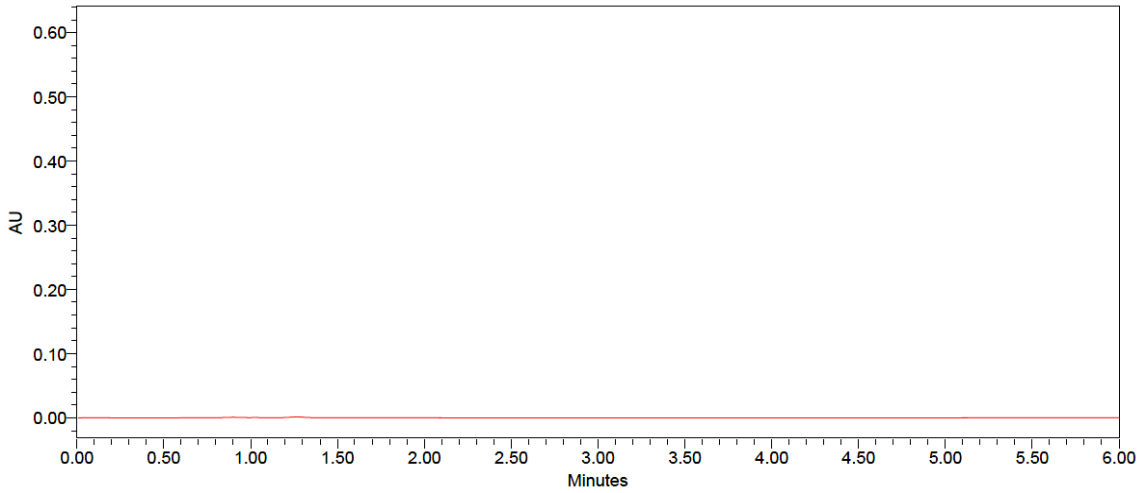

Figure 2. Chromatogram of blank

Linearity

By using a calibration curve to determine the linearity of the area of peak, its corresponding concentration was discovered. From this graph, it appears that the range of 25-150 µg/mL of Escitalopram and 1.25-7.5 µg/mL of Flupentixol had a straight line. Linearity results were demonstrated in table 3.

Table 3. Results of linearity

S. No	Escitalopram		Flupentixol	
	Concentration (µg/mL)	Area	Concentration (µg/mL)	Area
1	25.00	1329639	1.25	69046
2	50.00	2359735	2.50	120773
3	75.00	3394721	3.75	172648
4	100.00	4632381	5.00	230091
5	125.00	5729283	6.25	286974
6	150.00	7064333	7.50	351696

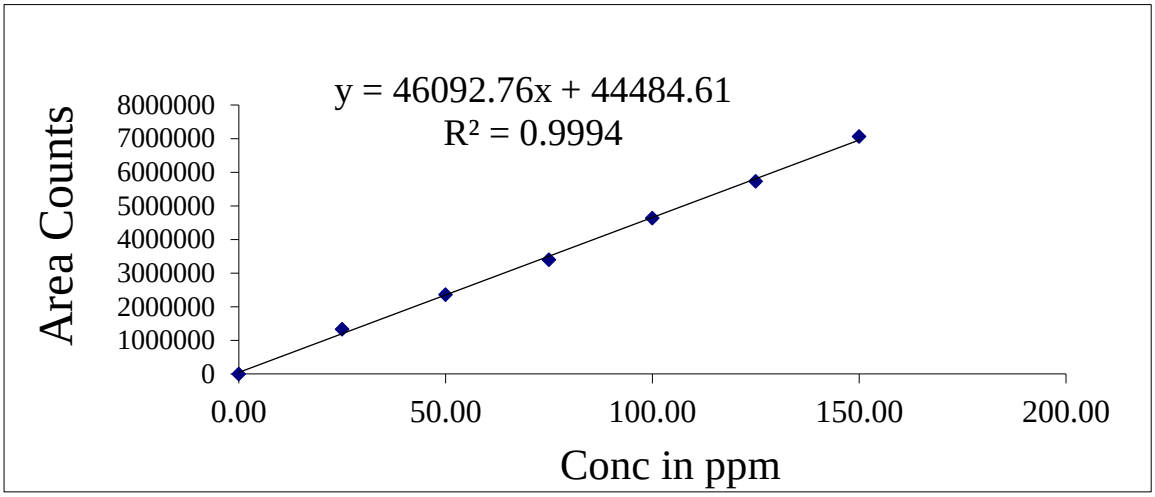

Figure 3. Calibration plot of Escitalopram

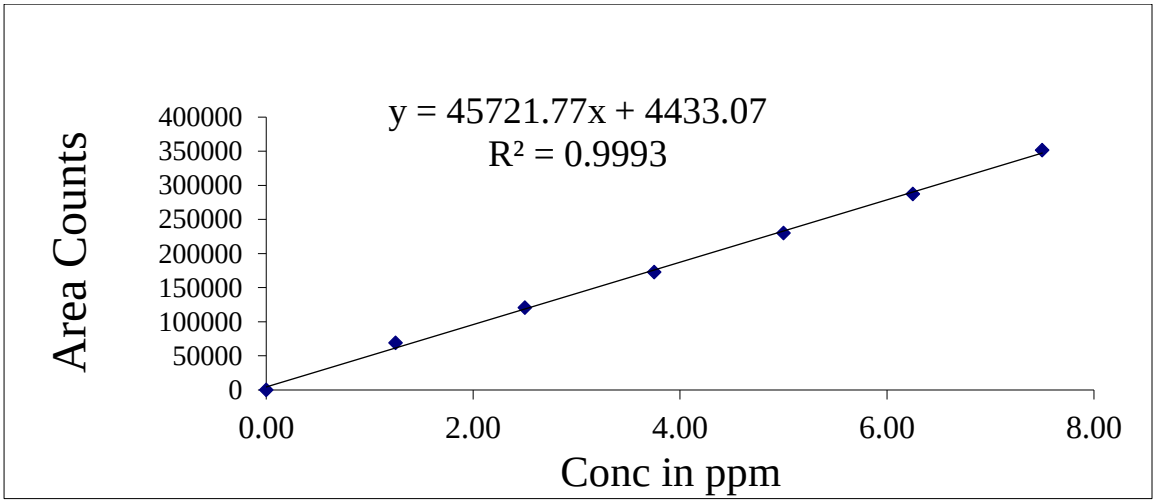

Figure 4. Calibration plot of Flupentixol

Precision

Intraday and intermediate precision variances were assessed in relation to the procedure's accuracy. The samples were examined six times on the same day to obtain intraday results for Flupentixol and Escitalopram. The system's intermediate precision was explored by analyzing data in the same laboratory using a variety of examiners and tools. It is very accurate, with an RSD percentage of less than 2%. The process was precise, yielding the best drug recoveries at each additional concentration. Table 4 shows the method precision results.

Table 4. Outcomes of method precision

S. No.	Escitalopram		Flupentixol	
	Area	% Assay	Area	% Assay
1	4702261	101.4	234747	101.2
2	4654589	100.4	231866	100
3	4625473	99.7	230541	99.4
4	4573675	98.6	232729	100.3
5	4670969	100.7	231376	99.7
6	4685239	101	232263	100.1
Mean	4652034	100.3	232254	100.1
Std. dev	46553.21	1.012	1435.03	0.618
% RSD	1.001	1.01	0.618	0.62

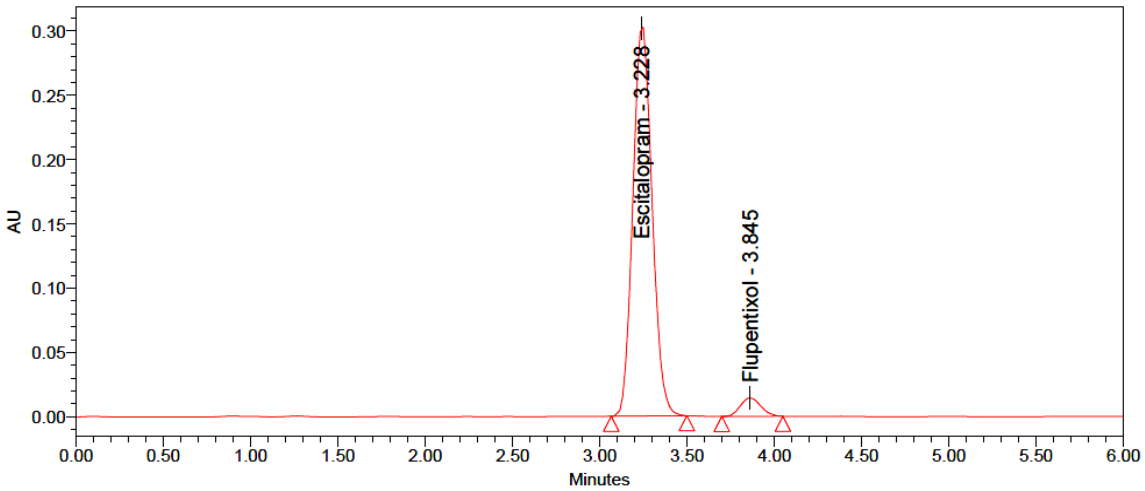

Figure 5. Chromatogram of method precision

Intermediate Precision (Ruggedness)

Intermediate precision results were shown in table 5.

Table 5. Results of intermediate precision

S.No.	Escitalopram		Flupentixol	
	Area	% Assay	Area	% Assay
1	4652261	100.2	234388	100.8
2	4699950	101.3	232178	99.8
3	4591334	98.9	234878	101.0
4	4606891	99.3	232064	99.8
5	4618068	99.5	235394	101.2
6	4706522	101.4	234768	100.9
Mean	4645838	100.1	233945	100.6
Std dev	48807.05	1.056	1449.42	0.621

% RSD	1.051	1.05	0.62	0.62

Accuracy

By measuring the recovery experiments at three stages, the method's precision was reached (50 percent, 100 percent, and 150 percent). APIs were made with concentrations of Flupentixol of 2.5, 5, and 7.5 micrograms/mL and Escitalopram of 50, 100 and 150 micrograms/mL. For each stage of the spike, the test solution was injected three times, and the assay was performed in accordance with the test process. In addition to being able to determine the percentage of recovered data, the mean and relative standard deviations have also been found. The strategy was effective because the recovery values fell within the target range. Table 6 presents the accuracy results.

Table 6.Results of accuracy

Accuracy	Amount of Escitalopram	% Recovery	Amount of Flupentixol	% Recovery
50*****	50	100.3	2.5	100.9
100*	100	100.5	5	100.4
150*	150	100.4	7.5	99.8

* Results are mean recovery of three sample preparations

LOD and LOQ

The concentration level at which the analytes are reliably detected and quantified is the limit of detection and quantification. Escitalopram and Flupentixol had a LOD concentrations of 0.3 µg/ml, 0.015 µg/ml and their S/N values of 3, 3. The LOQ concentrations of Escitalopram and Flupentixol were 1 µg/ml, 0.05 µg/ml, and their S/N values were 10, 10. (S/N is the ratio of signal to noise).

Robustness

To ensure the robustness of the chromatographic technique, the researchers evaluated flow rate and the composition of the mobile phase. By changing the flow rate and mobile phase ratio, the area of drugs changes. So, the percentage of relative standard deviation changes. Here in Table 7 (robustness results) the %RSD values are in within the acceptable limit.

Table 7. Outcomes of robustness

Parameter	% RSD of Escitalopram	% RSD of Flupentixol
Flow (0.8 mL/min)	0.98	1.51
Flow (1.2 mL/min)	1.1	0.74
Organic phase (77:23)	0.09	1.86
Organic phase (63:37)	0.41	1.10

Forced Degradation

The proposed approach can be used for successful evaluations of release and stability tests, and it can be called a stability preferable technique. Acid, Alkali, oxidation, reduction, photo, and thermal degradation are all included in the ICH-required forced degradation analysis. The chromatograms show that the selected drugs remained stable under the stress conditions, despite the presence of degraded peaks. Results of forced degradation were given in table 8.

Acid degradation

A volume of 1 ml sample stock solution was transferred to a volumetric flask with a capacity of 10 ml, to which 1 ml of 1N HCl was added and left to stand for 15 minutes. After 15 min add 1 ml of 1N NaOH and make up to the diluent mark. Filter the solution using syringe filter and injected into HPLC system.

Alkali degradation

A volume of 1 ml sample stock solution was transferred to a volumetric flask with a capacity of 10 ml, 1 ml of 1N NaOH was added, and the mixture was left to stand for 15 minutes. After 15 minutes, add 1 mL of 1N HCl to bring the solution up to the required concentration. Use a syringe filter to filter the solution, which will then be injected into the HPLC system.

Peroxide degradation

A volume of 1 ml sample stock solution was moved to a volumetric flask of 10 ml, add 1 ml of 30% hydrogen peroxide solution and make up to the mark with diluents. Filter the solution using syringe filter and injected into HPLC system.

Reduction degradation

Using a volumetric flask with a capacity of 10 ml, transfer 1 ml of sample stock solution and add 1 ml of 30% hydrogen peroxide solution, then dilute to the required concentration with diluents. Use a syringe filter to filter the solution, which will then be injected into the HPLC system.

Thermal degradation

During the 6-hour baking period, the sample solution was kept at 105°C. The resulting solution was injected into an high-performance liquid chromatography system.

Photolytic degradation

A weight of 100mg sample was exposed to sunlight for 6 hrs. and the exposed sample was analyzed. Prepare the sample solution by using this sample and inject into HPLC system.

Table 8. Forced degradation results

Stress Parameter (24 hrs)	% Degradation	
	Escitalopram	Flupentixol
Acid degradation (1N HCl)	14.5	12.7
Alkali degradation (1N NaOH)	12.6	13.4
Peroxide degradation (30% Peroxide)	15.1	13.6
Reduction degradation (30% sodium bi sulphate)	10.5	9.7
Thermal (sample, 70°C, 6 Hrs)	2.9	3.1
Photo (UV-Vis light- (200 W h/m^2) and fluorescent light (1.2 milliion lux-h)	4.2	3.6

4. Conclusion

In this study, a novel, quick, sensitive, and easy-to-use HPLC method was developed for the simultaneous estimation of Escitalopram and Flupentixol in API and pharmaceutical dosage types. Shorter run time, low cost, and all the other characteristics are benefits. All the parameters were verified and were found to be within the acceptable range, including linearity, accuracy, specificity, robustness, and process precision. According to our research, the RSD values for all the parameters came in at less than 2%, showing that the procedure is accurate and that the results we found are consistent. Therefore, it's possible to use the current approach in QC laboratories for routine study and manufacturing Flupentixol and Escitalopram pharmaceuticals without having to separate the substances first.

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