STRESS DEGRADATION STUDIES FOR SIMULTANEOUS ESTIMATION OF RESVERATROL AND GALLIC ACID BY RP-HPLC

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Abstract: *A stability indicating reversed- phase HPLC method has been developed for simultaneous estimation of gallic acid and resveratrol. The proposed RP-HPLC method utilizes a Nucleodur C_{18} having dimensions 5µ (250×4.6 mm) column, mobile phase consisting of Phosphate buffer pH 3 ±0.02 pH adjusted with ortho phosphoric acid and methanol and acetonitrile in the proportion of 50: 30: 20 v/v and UV detection at 263 nm and 301 nm for gallic acid and resveratrol, respectively using a shimadzu SPD-10AVP UV-Visible detector. Gallic acid and Resveratrol were exposed to acidic, basic, thermal, photolytic and oxidative stress conditions and stressed samples were analysed by the proposed method.*

Keywords: *Resveratrol, Gallic acid, Forced degradation study, stress testing.*

1. INTRODUCTION:

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a stilbenoid (Fig. 1.1) [1]. It is white powder with slight yellow cast, practically very slightly in water, but soluble in organic solvents such as methanol, acetone, DMSO, acetonitrile etc. It has molecular formula $C_{14}H_{12}O_3$ and molecular weight 228.25 with a melting point of 261 to 263 ºC. It has intracellular antioxidant activity [2] and activates SIRT1, a NAD^+-dependent histone deacetylase involved in mitochondrial biogenesis and the enhancement of peroxisome proliferator-γ-activated receptor coactivator-1α (PGC-1α) and FOXO activity [9-10]. The anti-diabetic, neuroprotective and anti-adipogenic actions of resveratrol may be mediated via SIRT1activation [7-8].

Gallic acid (also known as 3,4,5-trihydroxybenzoic acid) is a trihydroxybenzoic acid, a type of phenolic acid (Fig. 1.2) [3]. It is white, yellowish-white, or pale fawn- colored crystals, soluble in alcohol, ether, glycerol, acetone negligible in benzene, chloroform, petroleum ether [11]. It has molecular formula of $C_7H_6O_5$ and molecular weight 170.12 with a melting point of 260 ºC. Gallic acid is a water soluble phenolic acid present in grapes and in the leaves of many plants [4]. Gallic acid esters, such as tannins, catechin gallates and aliphatic gallates are potent antioxidants in vitro [5]. However, gallic acid itself also appears to have antioxidant, anticarcinogenic and antiangiogenic activity in vitro [6].

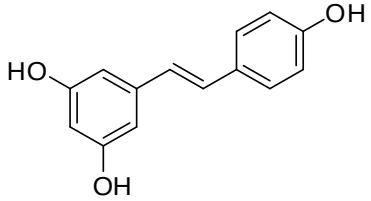

Fig. 1.1: Chemical structure of Resveratrol

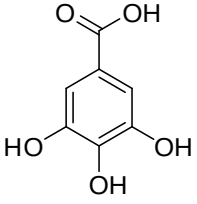

Fig. 1.2: Chemical structure of Gallic acid

Literature survey revealed that there is no method has been reported or published that is extensively focused on stress stability studies for simultaneous estimation of the gallic acid and resveratrol. Therefore, the aim of the present work is to establish the inherent stability of gallic acid and resveratrol through forced degradation studies. Aim of stability testing is to prove how the quality of a drug substance or drug product changes with time under the influence of a variety of factors such as excipients, temperature, pH, oxygen, light etc. Thus, stress studies are required in order to generate the method development, and its validation.

2. MATERIAL AND METHOD:

The drug Resveratrol (RES) was gifted from Lupin laboratories [Aurangabad, Bihar] and Gallic acid was procured as a gift sample from Symbiosis pharmaceuticals private limited [Sirmor, H.P.]. Acetonitrile [HPLC grade] and triethylamine were procured from Thermo fisher scientific india private limited [Mumbai] and glacial acetic acid purchased from Fischer scientific [Mumbai]. Orthophosphoric acid was procured from Thermo fisher scientific india private limited [Mumbai], hydrochloric acid (Merck, India), sodium hydroxide (Loba Chem, Mumbai) and hydrogen peroxide (Loba Chem, Mumbai) were used for analytical purposes.

2.1 HPLC Instrumentation and Chromatographic Parameters:

The chromatographic system used for the investigation was on shimadzu LC-2010 ATVP prominence liquid chromatograph and using shimadzu SPD-10AVP UV-Visible detector composed of binary pump, degasser, auto injector, and column oven. The chromatographic analysis was performed on a Nucleodur C_{18} having dimensions 5µ (250×4.6 mm) column. The mobile phase was a consisting of Phosphate buffer pH 3 ±0.02, pH adjusted with ortho phosphoric acid and methanol and acetonitrile (50: 30: 20 v/v), pumped at a flow rate of 1mL/min. The column temperature was maintained at 40°C, and the detection wavelength were 306 nm and 263 nm for Resveratrol and Gallic acid, respectively. Measurements were made with injection volume 20 µL and the run time was 7 min for each injection of stressed sample.

2.2 Preparation of buffer solution:

The buffer solution was prepared by dissolving 7.0g of potassium di hydrogen ortho phosphate in 1000ml of HPLC grade water and pH 3.0 was adjusted with orthophosphoric acid. It was filtered through 0.45µm nylon membrane filter and degassed with sonicator.

2.3 Preparation of blank solution:

Acetonitrile and methanol in ratio of 50: 50 was used as blank solution.

2.4 Preparation of standard solution:

The quantity of powder equivalent to 10 mg of resveratrol and gallic acid were weighed and transferred into 10 ml volumetric flask , 5 ml of diluent was added and sonicated for 15 minutes and the volume was made upto the mark with diluent. From this further dilution was made to get the final concentrations of resveratrol and gallic acid.

2.5 Stress degradation studies of Gallic acid and Resveratrol:

Intentional degradation was attempted to stress conditions of UV degradation, photolytic degradation, acid hydrolysis (using HCl), base hydrolysis (using NaOH) and oxidative degradation (using H2O2). Drug concentration of 1 mg/ml was used in all the degradation studies. After completion of the degradation processes, the solutions were neutralized and diluted with mobile phase.

2.5.1 Acid hydrolysis:

Sample solution containing 1 ml aliquot of mixture of both drugs was transferred into a 10 ml of amber volumetric flask, then mixed with 1 ml of 0.1M HCl and left to stand for 1 hr, 2 hr, 4 hr at 60°C ± 2°C after heating on bath samples were neutralized with 1 ml 0.1M NaOH and diluted up to 10 ml with diluents, sonicated and filtered through 0.22 µm membrane filter paper and injected in to HPLC system. All determinations were conducted in triplicate

2.5.2 Basic hydrolysis:

Sample solution containing 1 ml aliquot of mixtures of both drugs was transferred into a 10 ml of amber volumetric flask, then mixed with 1 ml of 0.1M NaOH and left to stand for 1 hr, 2 hr, 4 hr at 60°C ± 2°C after heating on bath samples were neutralized with 1 ml 0.1M HCl and diluted up to 10 ml with diluents, sonicated and filtered through 0.22 µm membrane filter paper and injected into HPLC system. All determinations were conducted in triplicate.

2.5.3 Oxidative degradation:

Sample solution containing 1 ml aliquot of mixture of both drugs was transferred into a 10 ml amber volumetric flask, then mixed with 1 ml of 1% (v/v) hydrogen peroxide and left to stand for 1 hr, 2 hr and 4 hr at 60 °C ± 2°C after heating on bath samples were diluted up to 10 ml with mobile phase. All three solutions were injected in triplicate.

2.5.4 Thermal degradation:

One milliliter aliquot of a sample solution containing mixture of both drugs was transferred to a 10 ml amber volumetric flask and then heated for 1 hr, 2 hr and 4 hr at 60 °C ± 2 °C. The resultant each of solution were diluted in mobile phase up to 10 ml and injected in triplicate.

2.5.5 UV degradation:

Photolytic degradation was studied by placing a mixture of both solution in a clear volumetric flask and exposing it to direct UV light (254nm) for 1 hr, 2 hr and 4 hr. The resultant solution was injected in triplicate.

3. RESULTS AND DISCUSSION:

3.1 Degradation in Acid:

The chromatogram for acid degradation of gallic acid dose not showed any significant degradation or additional peak of sample after 1 hr, 2 hr and 4 hr at 60 °C ± 2 °C degradation study (Figure 3.1). Resveratrol gradually decreased with time.

Table 3.1: Acid degradation of Gallic acid

Conditions	Conc. (µg/ml)	Time period	Peak area		Mean		% Degradation
			Before	After	Before	After	
Acid degradation	5	1 hr	188216	179259	187615.7	184421	1.7027718
			184653	185212			
			189978	188792			
	5	2 hr	188216	184218	187615.7	184396.7	1.71574158
			184653	184516			
			189978	184456			
	5	4 hr	188216	182408	187615.7	182307.7	2.82918804
			184653	182320			
			189978	182195			

Table 3.2: Acid degradation of Resveratrol

Conditions	Conc. (µg/ml)	Time period	Peak area		Mean		% Degradation
			Before	After	Before	After	
Acid degradation	5	1 hr	731859	53231	732355	36481.33	95.0186271
			731831	33897			
			733375	22316			
	5	2 hr	731859	17444	732355	17134.33	97.6603787
			731831	16567			
			733375	17392			
	5	4 hr	731859	7344	732355	7270.667	99.007221
			731831	7144			
			733375	7324			

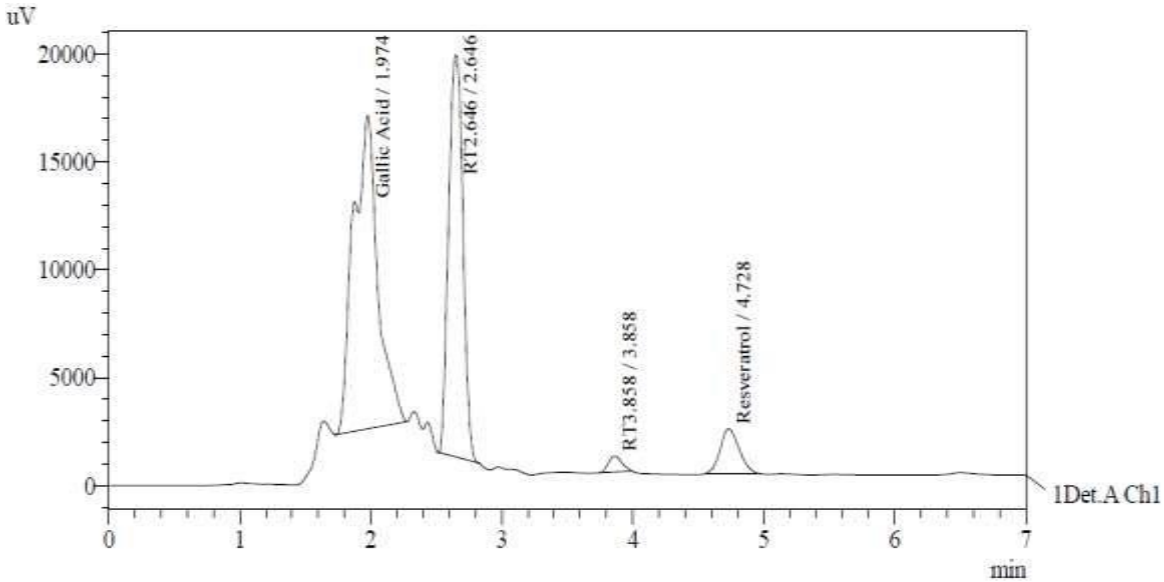

Fig. 3.1: Acid degradation

3.2. Degradation in Alkali:

The chromatogram for base degradation of Gallic acid do not showed any significant degradation of sample after 1 hr, 2 hr and 4 hr at 60 °C ± 2 °C degradation study but Resveratrol shows significant degradation after 1 hr, 2 hr and 4 hr at 60 °C ± 2 °C degradation study. (Figure 3.2)

Table 3.3: Base degradation of Gallic acid

Conditions	Conc. (µg/ml)	Time period	Peak area		Mean		% Degradation
			Before	After	Before	After	
Basic degradation	5	1 hr	188216	185198	187615.7	185150.7	1.31385616
			184653	185256			
			189978	184998			
	5	2 hr	188216	183965	187615.7	183695	2.08973309
			184653	183512			
			189978	183608			
	5	4 hr	188216	181981	187615.7	181775	3.11310178
			184653	181625			
			189978	181719			

Table 3.4: Base degradation of Resveratrol

Conditions	Conc. (µg/ml)	Time period	Peak area		Mean		% Degradation
			Before	After	Before	After	
Basic degradation	5	1 hr	731859	57680	732355	57663.67	92.1262685
			731831	57562			
			733375	57749			
	5	2 hr	731859	55584	732355	55613	92.4062784
			731831	55960			
			733375	55295			
	5	4 hr	731859	43005	732355	43451.67	94.0668574
			731831	43045			
			733375	44305			

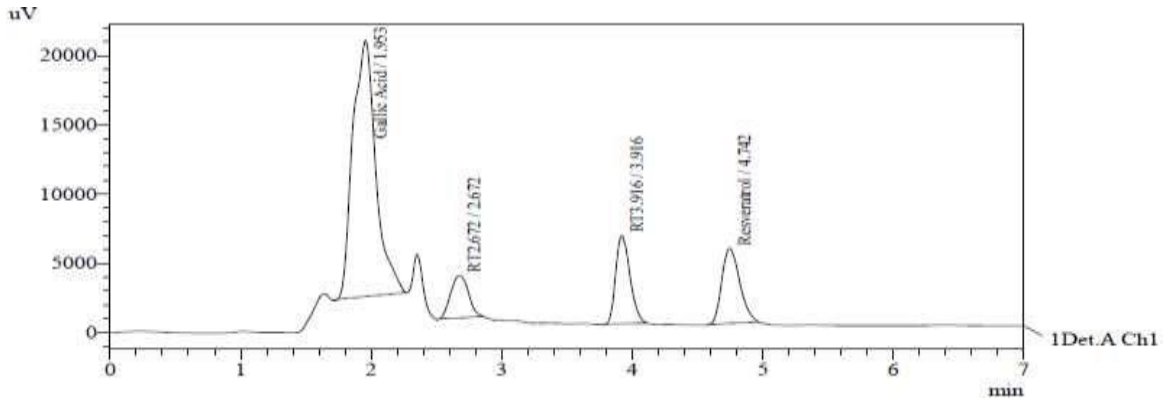

Fig. 3.2: Base degradation

3.3 Oxidative Stress:

The chromatogram for oxidative degradation of both drugs showed significant degradation in area, symmetry and additional peaks after 1 hr, 2 hr and 4 hr at 60 °C ± 2 °C of oxidative degradation study (Figure 3.3).

Table 3.5: Oxidative degradation of Gallic acid

Conditions	Conc. (µg/ml)	Time period	Peak area		Mean		% Degradation
			Before	After	Before	After	
Oxidative Degradation	5	1 hr	188216	39692	187615.7	39451.33	78.9722607
			184653	39850			
			189978	38812			
	5	2 hr	188216	37628	187615.7	37072.33	80.2402784

			184653	37047			
			189978	36542			
	5	4 hr	188216	32282	187615.7	31745.33	83.0795936
			184653	31729			
			189978	31225			

Table 3.6: Oxidative degradation of Resveratrol

Conditions	Conc. (µg/ml)	Time period	Peak area		Mean		% Degradation
			Before	After	Before	After	
Oxidative degradation	5	1 hr	731859	221573	732355	215553	70.567143
			731831	214794			
			733375	210292			
	5	2 hr	731859	123607	732355	120844.7	83.4991682
			731831	120510			
			733375	118417			
	5	4 hr	731859	5340	732355	5445.667	99.2564171
			731831	5512			
			733375	5485			

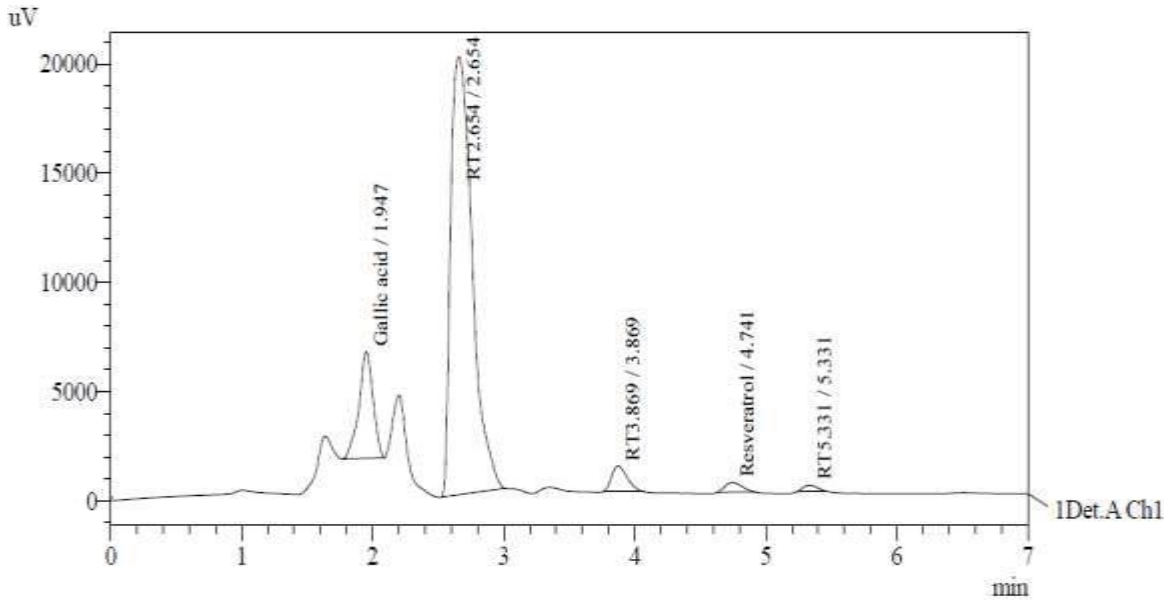

Fig. 3.3: Oxidative degradation

3.4 Thermal Stress:

The chromatogram for thermal degradation of Gallic acid do not showed any significant degradation or additional peak of sample after 1 hr, 2 hr and 4 hr at 60 °C ± 2 °C degradation study but Resveratrol shows significant degradation after 1 hr, 2 hr and 4 hr at 60 °C ± 2 °C degradation study. (Figure 3.4)

Table 3.7: Thermal degradation of Gallic acid

Conditions	Conc. (µg/ml)	Time period	Peak area		Mean		% Degradation
			Before	After	Before	After	
Thermal Degradation	5	1 hr	188216	187601	187615.7	187600.7	0.00799507
			184653	187425			
			189978	187776			
	5	2 hr	188216	185002	187615.7	185215.3	1.27938854
			184653	185212			
			189978	185432			
	5	4 hr	188216	183639	187615.7	183082.3	2.4162872
			184653	182408			
			189978	183200			

Table 3.8: Thermal degradation of Resveratrol

Conditions	Conc. (µg/ml)	Time period	Peak area		Mean		% Degradation
			Before	After	Before	After	
Thermal degradation	5	1 hr	731859	376192	732355	376240.3	48.6259624
			731831	376531			
			733375	375998			
	5	2 hr	731859	285018	732355	285087.3	61.0725218
			731831	285315			
			733375	284929			
	5	4 hr	731859	217139	732355	217374	70.3184931
			731831	217591			
			733375	217392			

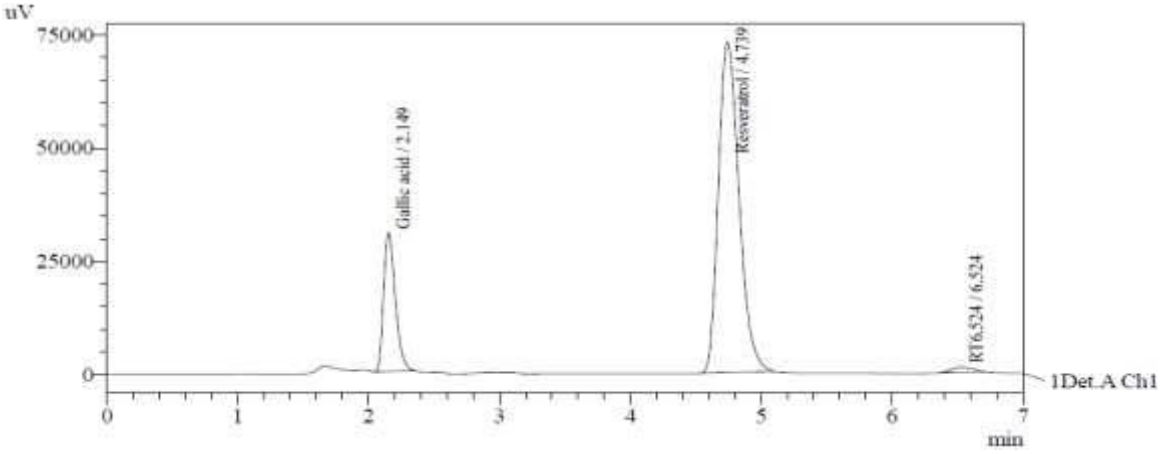

Fig. 3.4: Thermal degradation

3.5 UV degradation:

The chromatogram for UV degradation of Gallic acid do not showed any significant degradation or additional peak of sample after 1 hr, 2 hr and 4 hr at 60 °C ± 2 °C degradation study but Resveratrol shows significant degradation after 1 hr, 2 hr and 4 hr at 60 °C ± 2 °C degradation study. (Figure 3.5)

Table 3.9: UV degradation of Gallic acid

Conditions	Conc. (µg/ml)	Time period	Peak area		Mean		% Degradation
			Before	After	Before	After	
UV degradation	5	1 hr	188216	184912	187615.7	184369.7	1.7301327
			184653	184198			
			189978	183999			
	5	2 hr	188216	182468	187615.7	182406.3	2.77659826
			184653	182191			
			189978	182560			
	5	4 hr	188216	180108	187615.7	180289.3	3.90496885
			184653	180512			
			189978	180248			

Table 3.10: UV degradation of Resveratrol

Conditions	Conc. (µg/ml)	Time period	Peak area		Mean		% Degradation
			Before	After	Before	After	
UV degradation	5	1 hr	731859	384031	732355	385666.7	47.3388361
			731831	386706			
			733375	386263			

	5	2 hr	731859	284028	732355	284109	61.2061091
			731831	284062			
			733375	284237			
	5	4 hr	731859	217139	732355	216852.3	70.3897245
			731831	216705			
			733375	216713			

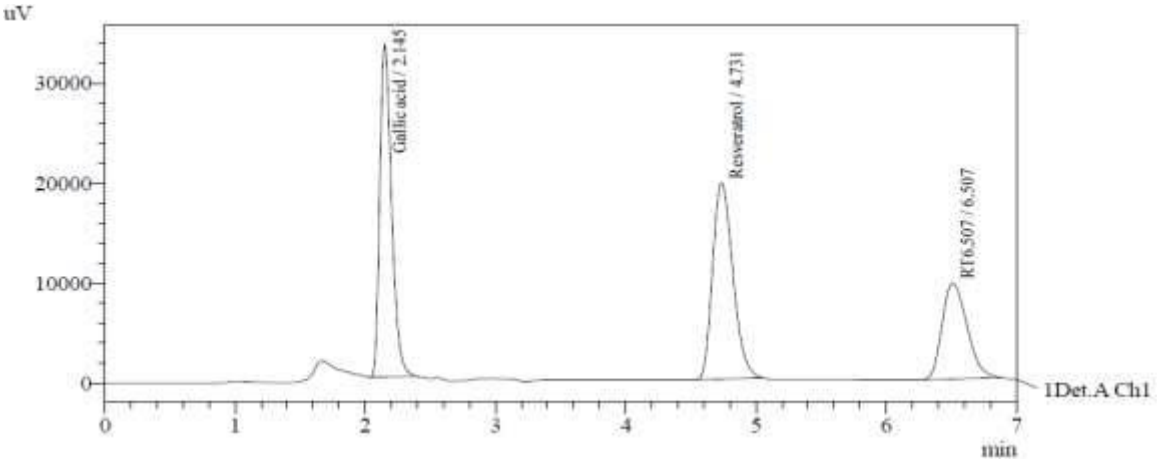

Fig. 3.5: UV degradation

4. CONCLUSION:

Forced degradation studies revealed that reveratrol prone to degraded under all the performed conditions. Moreover, the content of degradation of gallic acid and resveratrol in various conditions, such as alkaline, acidic, oxidation, thermal and photolytic, were observed and quantitatively analyzed by this HPLC method. The information, thus, obtained will facilitate pharmaceutical development in areas such as formulation development, manufacturing, and packaging, where knowledge of chemical behavior can be used to improve the quality of drug product.

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