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A validated reverse phase HPLC and HPTLC method for estimation of Olmesartan Medoxomil in pharmaceutical dosage form

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ABSTRACT

Two novel chromatographic methods having requisite precision, accuracy, specificity and robustness were developed and validated for quantitative determination of Olmesartan medoxomil (OLM) in pharmaceutical dosage forms. The first method was based on isocratic reverse phase liquid chromatography using promosil RP C18 column (250 x4.6mm ID,5µm) and mobile phase consists of Methanol : 25 mM Phosphate buffer (70:30 v/v; pH = 4.6) at a flow rate of 1ml/min and detection was achieved with photodiode array detector set at 256nm.The response was linear over a range of 20-140µg/ml (R^2=0.9996). The second method involves precoated silica gel 60F_{254} High performance thin layer chromatography with densitometric detection at 256nm using Chloroform: Acetone: Methanol (7:2:1v/v/v) as mobile phase. The calibration curve ranges between 200-800 ng/spot(R^2=0.9984). Validation of method was carried out fulfilling ICH guidelines (Q2R1). Both the methods were applied without any interference from excipients, for determination of drug in coated tablets. It is suggested that the proposed HPLC and HPTLC procedure could be used for routine quality control and dosage form assay of OLM.

Key words: Olmesartan medoxomil (OLM), RP-HPLC, HPTLC, Validation

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INTRODUCTION

Olmesartan Medoxomil(OLM) [5-methly-2-oxo-1,3-dioxol-4-yl]methyl ester of 4[1-hydroxy-1-methyl ethyl]-2-propyl-1-{[2'-[1H-tetrazol-5-yl][1,1'-Biphenyl]-4-yl]-1H-imidazole-5-caboxylic acid} is prescribed as an antihypertensive and classified as an selective AT1 subtype angiotensin II receptor antagonist [1].

This sartan is an ester prodrug that is hydrolysed during absorption from gastrointestinal tract to active form olmesartan by arylesterase [2].

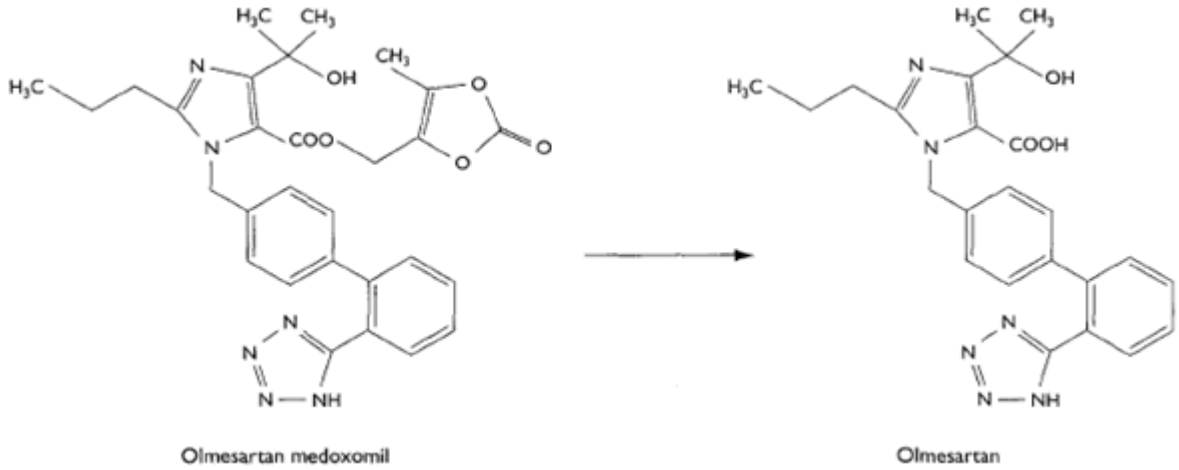

Figure 1: Olmesartan Medoxomil and its active metabolite Olmesartan

OLM has not being described in any official compedias. Literature survey revealed that several analytical methods were reported for determination of OLM in biological fluid using Liquid chromatography coupled to flouroscence detector, tandem mass spectrometry [3-5].There are also reports on quantitative spectrophotometeric determination of this drug in coated tablet [6], and even on simultaneous determination of OLM and hydrochlorothaizde in tablet dosage form using liquid chromatography [7].

No method using HPTLC have being reported yet. In the present investigation an attempt has been made to develop an economically viable RP-HPLC method which is better than other HPLC methods found in literature and a novel HPTLC method, both of which are validated as per ICH guidelines (Q2R1), meeting required criteria for specificity, accuracy and precision and will even be reproducible and suitable for routine quality control analysis of drug in pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals:

OLM was received as a kind gift sample from Ranbaxy Laboratories (Gurgaon, Haryana). All chemicals were HPLC grade. Methanol, Acetone (Lobechemia), Chloroform (Merck), Potassium dihydrogen ortho phosphate-LR grade (CDH). HPLC grade water was taken from MilliQ water purification system (Millpore). Commercially available tablets of Olvance^{TM} (Ranbaxy lab) containing 40 mg of drug was used for analysis.

Instrumentation

HPLC: Chromatographic separation was performed on a Waters liquid chromatographic system equipped with a Waters 515 HPLC pump, Waters 2998 photo diode array detector and Waters 717 plus autosampler with 10 µl syringe. Empower software was used for data collection and processing. Analytical promosil C18 column (250 x 4.6 mm ID, 5 µm) was used for separation purpose.

HPTLC: Camag HPTLC system comprising of Linomat5 automatic sample applicator, Hamilton syringe(100µl), camag TLC scanner3, Camag WinCATS software, Camag twin trough chamber(10x10) were used during study.

Stock solution of standard OLM for RP-HPLC and HPTLC
OLM stock solution (1mg/ml) was prepared in methanol which was further diluted with methanol to give concentration range of 20-140 µg/ml for HPLC analysis.

For HPTLC stock solution of 1 mg/ml and standard working concentration of 100 µg/ml was prepared using methanol so that a 200-800 ng/spot of OLM was spotted on precoated plates.

Chromatographic conditions
RP-HPLC
Measurement was performed isocratically using promosil RP C18 column (250 x4.6mm ID,5µm) operated at ambient temperature with mobile phase of Methanol: 25 mM Phosphate buffer (70:30 v/v; pH = 4.6) which was filtered using 0.45µm of membrane filter and degassed. The flow rate was adjusted to 1 ml/min and photodiode array detector was set at 256 nm. The injection volume was 10µl for both reference substance and the drug product.
HPTLC
Chromatography was performed on 10 x 10 cm precoated silica gel 60 F254 plates (Merck). Before use plates were washed with methanol and activated on TLC plate heater at 105 ºC for 5 min. Ascending development of plate with migration distance of 72mm was performed at ambient temperature using Chloroform: Acetone: Methanol(7:2:1 v/v/v) as mobile phase and Camag twin trough chamber previously saturated with mobile phase for 30 min.

Validation method
The developed HPLC and HPTLC methods were validated for specificity, linearity, precision, accuracy, robustness and system suitability following ICH guidelines [8].

Validation parameters
The developed HPLC method was validated for specificity, linearity, precision, robustness and system suitability following ICH guidelines.

Linearity
HPLC: The calibration curve was constructed by preparing methanolic solution of the drug with concentration range between 20-140µg/ml.

HPTLC: 2, 3, 4, 5, 6, 7, 8µl volumes of working standard were applied on HPTLC plate as separate spots of 200, 300, 400, 500, 600, 700, 800 ng/spot to cover the concentration range of 200-800 ng/spot.

Linearity was evaluated by linear regression analysis, which was calculated by least square regression method. Peak area v/s concentration was used for plotting linearity graph.

Limit of Detection (LOD) & Limit of Quantification (LOD)
LOD and LOQ were estimated from signal to noise ratio. The detection limit was determined as the lowest concentration level resulting in peak area of three times the baseline noise. The quantitation limit was determined as the lowest concentration level that provides a peak area with signal to noise 10.

Precision
Repeatability

HPLC: Intraday variation was seen. Three concentration (60, 80, 100µg/ml) were prepared near the test concentration (i.e 80 µg/ml), which were injected three times a day and area reported was obtained.

HPTLC: Intraday variation was seen by analyzing spots of three different concentration of OLM (400, 500, 600 ng/spot) in three replicate on single day using methanolic solution of 100 µg/ml.

The % RSD was calculated for the area thus obtained to get intraday variation

Intermediate precision: It was done on three different days using above mentioned three concentrations, for both HPLC and HPTLC.

Reproducibility : It was established by analyzing three different concentration on two different equipments and two different analyst.

Specificity

HPLC: Three different concentration of OLM (60, 80, 100 µg/ml) were prepared in methanol. Lactose, talc, starch, magnesium stearate, microcrystalline cellulose were added as excipients and compared with that of pure OLM solution of same strength. The t-test (paired, two tailed distribution) was applied to compare the result.

HPTLC: 400, 500, 600 ng/spot of standard drug solution was compared with standard drug solution spiked with excipients, which are present in marketed formulation.

Accuracy

HPLC: The recovery studies, also known as standard addition method, is performed by addition of known amount of the standard drugs to a solution of known concentration of previously analysed commercial pharmaceutical product.

The recovery studies were performed by adding 60, 80, 100µg/ml of solution of standard drug in previously analyzed solution of tablet.

HPTLC: Standard addition method was performed to support the accuracy by adding separately three different concentration of OLM (400, 500, 600ng/spot) to preanalysed OLM solution (Olvance^{TM}) of 500 ng/spot and analyzing them.

Robustness

HPLC: The robustness of method was checked by evaluating system suitability parameters data obtained after varying the HPLC pump flow rate (±5%), mobile phase composition (±5%), column temperature (±4 ºC).

HPTLC: Robustness of the proposed method was determined by changing the chamber saturation time (30±5min), detection wavelength.

System suitability test for HPLC

System suitability parameters like tailing factor, capacity factor, number of theoretical plates etc were calculated and compared with standard value.

Analysis of tablet formulation

HPLC

Twenty tablets were weighed, triturated and average tablet weight was calculated and portion of powder equivalent to 25mg of drug was accurately weighed and transferred to 25ml of volumetric flask. Drug was dissolved by adding methanol with constant stirring, followed by filtration through 0.45µm membrane filter. The final concentration of 80 µg/ml of OLM was prepared and its concentration was calculated using regression equation.

HPTLC

Twenty tablets were weighed, triturated and average tablet weight was calculated and portion of powder equivalent to 25 mg of drug was accurately weighed and transferred to 25 ml of volumetric flask containing methanol to give concentration of 1mg/ml and filtered though whatmann filter paper No 42. 1 ml of tablet stock was diluted to 10 ml with methanol to give final concentration of 100µg/ml. 5µl of this solution was spotted on HPTLC plates to give a concentration of 500 ng/spot of OLM.

For both HPLC and HPTLC the area under chromatogram was read and amount of drug was estimated by comparison with working standard using formulae

$$\% \text{ of Labelled claim} = \frac{AUC_{sample} \times \text{Average wt.of tablet} \times \text{Concentration}_{standard}}{AUC_{standard} \times \text{wt.of standard taken} \times \text{labeled claim}} \times 100$$

RESULTS AND DISCUSSION

Selection of chromatographic conditions

HPLC: The chromatographic condition were optimized after testing some important parameters like pH of the mobile phase, concentration of buffer solution, percentage and type of organic modifier, flow rate etc. Trails shows acidic pH give symmetric sharp peak so 25mM phosphate buffer of pH 4.6 was preferred as buffer. Methanol was chosen as organic modifier because it solves the drug very well with good retention time.
So satisfactory separation was obtained when using Methanol: 25 mM phosphate buffer of pH 4.6 (70:30 v/v) under isocratic condition, at flow rate of 1 ml/min using promosil RP C18 column.

A well define, sharp peak (almost free from tailing) was observed at a retention time of 4.4 min, when detected at 256nm.

HPTLC: A number of experimental parameters, such as mobile phase composition, scan mode, detection wavelength were optimized during method development.

A well resolved sharp peak at $R_F = 0.4$ with minimum tailing was obtained when using a mobile phase composition of Chloroform: Acetone: Methanol (7:2:1 v/v/v)

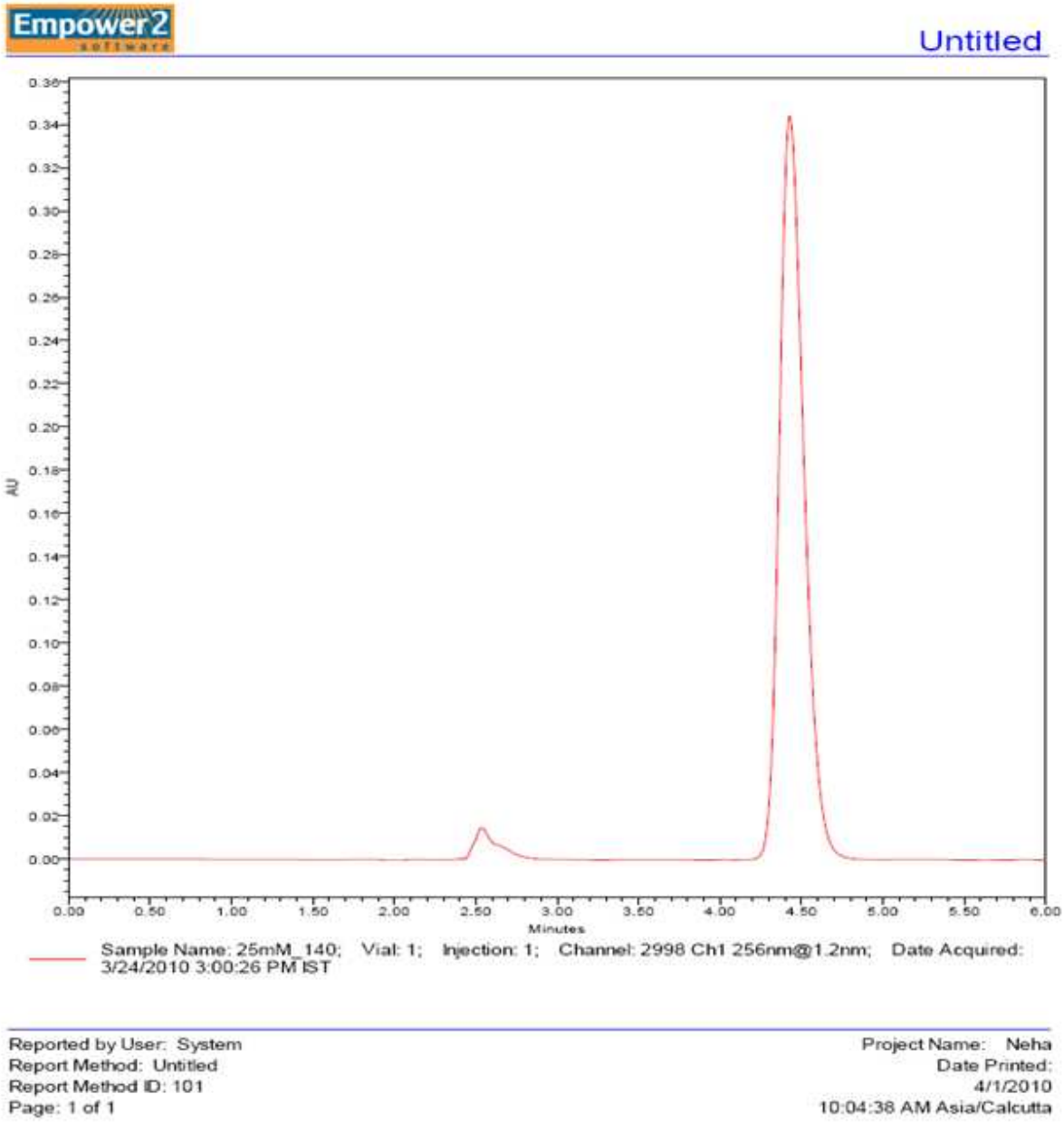

Figure 2: HPLC Chromatogram of OLM showing peak at Rt = 4.4 min

Validation

The developed HPLC and HPTLC method were validated for specificity, Linearity, precision, accuracy, robustness, system suitability (HPLC).

Linearity: (Table 1)

The calibration curves were prepared by plotting the area under curve (AUC) v/s concentration of drug.

HPLC: Linearity was observed in the range of 20-140µg/ml with mean regression equation.

$y = 25744x – 8225.6$ (R^2=0.9996)

HPTLC: Linearity was observed in range of 200-800 ng/spot with mean regression equation

$y = 7.0788x + 1197.7$ ($R^2 = 0.9970$)

y = AUC, x = Concentration of drug

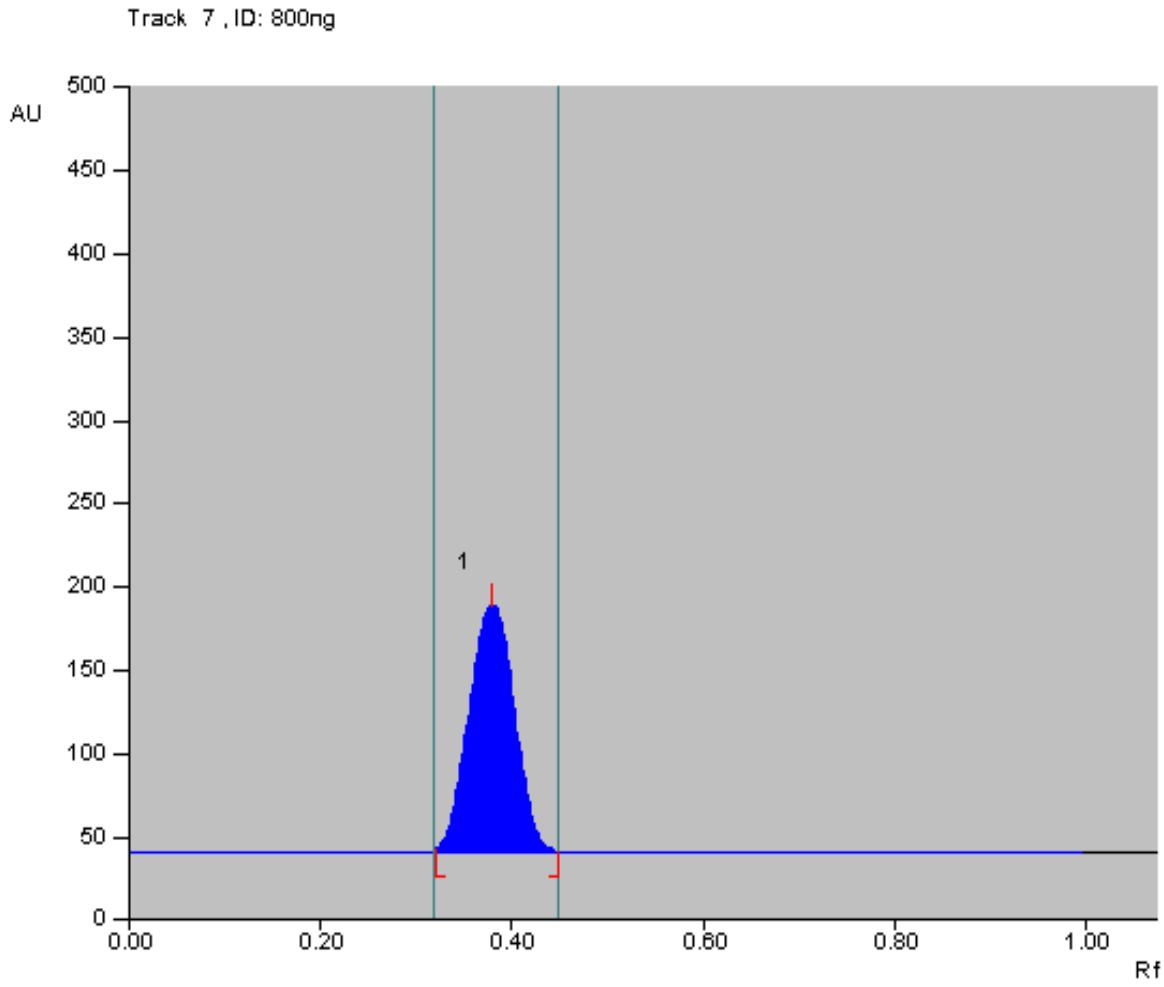

Figure 3:HPTLC Chromatogram of OLM showing peak at Rf =0.4

Table 1: Validation parameter for determination of OLM

VALIDATION PARAMETER	HPLC	HPTLC
LINEARITY RANGE	20-140µg/ml	200-800ng/spot
CORELLATION COEFFICIENT	0.9997	0.9984
SLOPE (m)	25744	7.0778
INTERCEPT (c)	8225.6	1197.7
LOD	2.96µg/ml	38.62ng/spot
LOQ	9.86µg/ml	128ng/spot

Precision: (Table 2)
Intraday precision was performed by relative standard deviation of 3 repeated assay of sample at 3 concentration level. Interday precision was determined by analysing sample thrice on three different days. The RSD were found to be 0.35-1.26% and 0.22-0.60 % respectively for HPLC and HPTLC method.

For HPLC analysis was done on Waters HPLC (with promosil RP C18 column) and Shimadzu HPLC (with Lichrospher RP C18 column) on three different concentration and results were reproducible with % RSD=1.14

Table 2: Intraday and Interday precision

Actual Concentration	Intraday Precision		Interday Precision	
	Found Mean Concentration (n = 3)	%RSD	Found Mean Concentration (n = 9)	%RSD
HPLC (µg/ml)				
60	61.75	0.44	61.24	0.89
80	80.96	0.35	80.33	1.26
100	100.3	0.55	100.56	0.81
HPTLC (ng/spot)				
400	402.13	0.60	401.40	0.50
500	501.6	0.24	501.20	0.24
600	602.1	0.28	601.90	0.22

Accuracy (Table 3)
The % recovery obtained as 98.47-99.50% for HPLC and 99.97-100.20% for HPTLC, indicating good accuracy of both the methods

Table 3: Result of accuracy studies by standard addition method

Concentration of drug taken	Amount of pure drug added	Total found concentration Mean (n=4)	% Recovery of pure drug added
HPLC (µg/ml)			
80	08	86.66	98.47
80	16	94.51	98.44
80	24	103.49	99.50
HPTLC (ng/spot)			
500	50	550.95	100.20
500	100	599.86	99.97
500	150	651.02	100.15

Specificity
Absence of any peak other than at R_t = 4.4 min in HPLC chromatogram and any other secondary spot than that of OLM at R_f = 0.4, confirms specificity of analytical methods.
Moreover the results obtained after applying t-test were within the acceptable limit.

Robustness
To ensure the insensitivity of the two methods to minor changes in experimental condition, it is important to demonstrate robustness of method, by modification in flow rate, detection wavelength, column temperature for HPLC and change in saturation time, detection wavelength etc for HPTLC. None of the method caused a significant change in resolution.

System suitability (Table 4)

Table 4: System suitability parameters (HPLC)

AUC	Retention time	Tailing factor	Assymetric factor	Theoretical plates/meter
3614795	4.428	1.00	1.00	11162.484

Tablet studies

The proposed method was successfully applied to the analysis of marketed product as demonstrated in Table 5.

Table 5: Application of validated method on marketed formulation

Formulation and method used	Label claim (mg/tablet)	Amount found (mg) (n=6)	%RSD	Recovery %
OLVANCE(HPLC)	40	39.62	0.58	99.06
OLVANCE(HPTLC)	40	39.6	0.43	99.00

CONCLUSION

Two precise, accurate, specific, robust and cost effective chromatographic methods, requiring simple reagents with minimum sample preparation were developed and statistical analysis proved that methods are reproducible and selective for quantitative determination of OLM in pharmaceutical dosage form.

Both RP-HPLC and HPTLC methods are suitable for routine analysis, as well as for the quality control of raw materials, formulation and dissolution studies.

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