HPTLC Method Development and Validation of Naproxen Sodium and Esomeprazole Magnesium Trihydrate in Combined Dosage Form

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Abstract: A simple, precise, specific and accurate HPTLC method has been developed and validated for combination of Naproxen Sodium and Esomeprazole Magnesium Trihydrate in pharmaceutical dosage form. The separation was carried out on HPTLC Aluminium plates pre-coated with silica-gel 60 F254(10 ×10 cm) using Ethyl acetate: toluene : methanol (4:5.5:0.5 v/v/v) as mobile phase. HPTLC separation of the two drugs followed by chromatographic measurement was carried out in the absorbance mode at 285 nm. The drugs were resolved satisfactorily with RF values of 0.73 ± 0.01 and 0.43 ± 0.01 for Naproxen Sodium and Esomeprazole Magnesium Trihydrate respectively. The linear regression analysis data for the calibration plots showed good linear relationship with R^2=0.999 and 0.998 for Naproxen Sodium and Esomeprazole Magnesium Trihydrate, respectively in the concentration range of 400-2400 ng/spot for Naproxen Sodium and 20-120 ng/spot for Esomeprazole Magnesium Trihydrate. The method was validated for accuracy, precision, specificity and robustness. The limit of detection and quantitation were 2678.72 ng/spot and 811.32 ng/spot, respectively for Naproxen Sodium and 126.00 and 388.68 ng/spot, respectively for Esomeprazole Magnesium Trihydrate. The proposed developed HPTLC method can be applied for identification and quantitative determination of Naproxen Sodium and Esomeprazole Magnesium Trihydrate in bulk drug and drug formulation.

Index Terms **- HPTLC, Naproxen Sodium, Esomeprazole Magnesium Trihydrate, Pharmaceutical formulations**

I. INTRODUCTION

Naproxen is a nonsteroidal anti-inflammatory drug (NSAID) used to relieve symptoms of arthritis (osteoarthritis, rheumatoid arthritis, or juvenile arthritis) such as inflammation, swelling, stiffness, and joint pain. It is a propionic acid derivative and a non-steroidal anti-inflammatory drug (NSAID) with anti-inflammatory, antipyretic and analgesic activities. Naproxen inhibits the activity of the enzymes cyclo-oxygenase I and II, resulting in a decreased formation of precursors of prostaglandins and thromboxanes. The resulting decrease in prostaglandin synthesis is responsible for the therapeutic effects of naproxen. Naproxen also causes a decrease in the formation of thromboxane A2 synthesis, by thromboxane synthase, thereby inhibiting platelet aggregation.[1-3]

Esomeprazole Magnesium is the magnesium salt of esomeprazole, the S-isomer of omeprazole, with gastric proton pump inhibitor activity. In the acidic compartment of parietal cells, esomeprazole is protonated and converted into the active achiral sulphenamide, the active sulphenamide forms one or more covalent disulfide bonds with the proton pump hydrogen-potassium adenosine triphosphatase (H+/K+ ATPase), thereby inhibiting its activity and the parietal cell secretion of H+ ions into the gastric lumen, the final step in gastric acid production. H+/K+ ATPase is an integral membrane protein of the gastric parietal cell.[1-3]

Combination of Naproxen Sodium and Esomeprazole Magnesium Trihydrate are used to treat long term arthritis with risk of NSAID causing peptic ulcer.

High Performance Thin layer Chromatography (HPTLC) is a sophisticated and automated form of the thin layer chromatography (TLC) with better and advanced separation efficiency and detection limits. It is also known as High Pressure Thin layer Chromatography/Planar chromatography or Flat-bed chromatography. It is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks. Separation may result due to adsorption or partition or by both phenomenon's depending upon the nature of adsorbents used on plates and solvents system used for development.[4-6]

II. Materials

Working Standards of Naproxen Sodium API was provided as a gift sample from Dr. Reddy's, Nalgonda Plant Peddadevulapally Tripuraram Mandal Nalgonda District, Telangana State, India and Esomeprazole magnesium Trihydrate API was provided as a gift sample from Cadila Health care, Vadodara.

III. Method

3.1 Selection of Analytical Detection Wavelength

The standard solution of Naproxen Sodium (60µg/ml) and Esomeprazole Magnesium Trihydrate (3µg/ml) in methanol was recorded using double beam UV spectrophotometer.

3.2 Apparatus

A CAMAG HPTLC system (Switzerland) comprising a CAMAG Linomat IV semiautomatic sample applicator, a CAMAG TLC Scanner 3, A CAMAG twin-trough chamber (10 × 10 cm), CAMAG CATS 4 software, A Hamilton syringe (100 µl) was used during the study.

3.3 Chromatographic condition

The chromatographic conditions were optimized and estimations were performed on a stationary phase, pre coated silica gel 60 F_{254} aluminium sheets (10 × 10 cm) which were pre-washed with methanol and dried in air, with mobile phase of Ethyl acetate: toluene : methanol (4:5.5:0.5 v/v/v) . The chromatographic chamber and plate was allowed to saturate for about 20 min and the migration distance allowed was 72 mm. The wavelength scanning was performed at 285 nm keeping the slit dimension 4.00 × 0.30 mm. The source of radiation was deuterium lamp emitting a continuous UV spectrum between 200-400nm. The standard solutions of Naproxen Sodium and Esomeprazole Magnesium Trihydrate was spotted and developed at constant temperature of 25 ± 2°C.

3.4 Preparation of mobile phase

Ethyl acetate: Toluene: methanol (4:5.5:0.5 v/v/v) was employed as mobile phase.

3.5 Preparation of standard solution of Naproxen Sodium and Esomeprazole Magnesium Trihydrate

Accurately weighed 400 mg of standard Naproxen Sodium was transferred to 100 ml volumetric flask, dissolved in 5 ml methanol and diluted up to the mark with same to get stock solution having strength 4000µg/ml for Naproxen Sodium.

Accurately weighed 20 mg of standard Esomeprazole Magnesium Trihydrate was transferred to 100 ml volumetric flask, dissolved in 5 ml methanol and diluted up to the mark with same to get stock solution having strength 200µg/ml for Esomeprazole Magnesium Trihydrate.

3.6 Preparation of calibration curve

Aliquots 1, 2, 3, 4, 5 and 6 µl of standard stock solution of 400 µg/ml of Naproxen Sodium and 1, 2, 3, 4, and 6µl of standard stock solution of 20 µg/ml of Esomeprazole Magnesium Trihydrate were spotted on pre-coated TLC plate under nitrogen stream using Linomat V semi-automatic sample applicator. The plate was dried in the air and developed up to 80 mm using mixture of Ethyl acetate: toluene : methanol 4:5.5:0.5 v/v as mobile phase in a twin trough chamber previously saturated with mobile phase for 30 minutes. The plate was removed from the chamber, dried in hot air oven and scanned and quantified at 285 nm in absorbance mode. The calibration curve was constructed by plotting area versus respective concentration (ng/spot).

3.7 Method validation

The developed method was validated as per ICH Q2 (R1) guidelines.

3.7.1 Linearity and range

The linearity was expressed in terms of correlation co-efficient of linear regression analysis. The linearity range was determined by analyzing 4 independent levels of calibration curve in the range of 400-2000ng/spot for Naproxen Sodium and 2 independent levels of calibration curve in the range of 20-100ng/spot for Esomeprazole Magnesium Trihydrate.

3.7.2 Specificity

The spot of Naproxen Sodium and Esomeprazole Magnesium Trihydrate from dosage form were confirmed by comparing its R_f and absorbance-reflectance spectrum with that of standard Naproxen Sodium and Esomeprazole Magnesium Trihydrate. The peak purity of Naproxen Sodium and Esomeprazole Magnesium Trihydrate from each sample was determined by comparing the spectra scanned at peak start, peak apex, and peak end positions of the spot.

3.7.3 Precision

3.7.3.1 Repeatability of Measurement

Standard stock solution of Naproxen Sodium 400 ng/spot and Esomeprazole Magnesium Trihydrate 20 ng/spot was spotted on a TLC plate, and analyzed by the proposed method. The obtained band was scanned seven times without changing plate position and percent RSD for measurement of peak area was calculated.

3.7.3.2 Repeatability of Sample Application

Standard solution 400 ng/spot of Naproxen Sodium and Esomeprazole Magnesium Trihydrate 20 ng/spot was spotted on a TLC plate seven times, and analyzed by the proposed method. The area of seven spots was measured and percent RSD of peak area was calculated.

3.7.3.3 Intra-Day and Inter-Day Precision

Variation of the results within same day is called intra-day precision and variation of results amongst days is called inter-day precision. Intra-day precision and Inter-day precision of the proposed method was evaluated by analyzing the range of Naproxen Sodium (1200, 1600 and 2000 ng/spot) and Esomeprazole Magnesium Trihydrate (60, 80 and 100 ng/spot), three times on same day and three different days respectively.

3.7.4 Accuracy

Known amount of standard Naproxen Sodium and Esomeprazole Magnesium Trihydrate was added at 80%, 100% and 120% level to pre-analyzed sample of Naproxen Sodium and Esomeprazole Magnesium Trihydrate. The quantity of tablet powder equivalent to 375 mg of Naproxen Sodium and 20 mg of Esomeprazole Magnesium Trihydrate was transferred to four individual 100 ml volumetric flasks. Known amount of standard Naproxen Sodium and Esomeprazole Magnesium Trihydrate was spiked to pre-analyzed sample of Naproxen Sodium and Esomeprazole Magnesium Trihydrate according to table 3.1. 10 ml methanol was added to dissolve powder drug sample was made up to mark with methanol. Filter and Pipette out 2.5 ml and dilute upto 25 ml with methanol. A volume of 2 µl of all the solutions were spotted and analyzed.

Table: 1 Preparation of solutions for Accuracy of Naproxen Sodium and Esomeprazole Magnesium Trihydrate

Level	Naproxen Sodium			Esomeprazole Magnesium Trihydrate		
	Amount sample	Amount STD spiked	Total amount (mg)	Amount sample	Amount STD spiked	Total amount (mg)
0%	375	-	375	20	-	20
80%	375	300	675	20	16	36
100%	375	375	750	20	20	40
120%	375	450	825	20	24	44

3.7.5 Limit of Detection

Limit of detection was calculated using following equation as per ICH guidelines. LOD= 3.3 x SD/S

Where SD is the standard deviation of the Y- intercepts of the six calibration curves and S is mean slope of the five calibration curves.

3.7.6 Limit of Quantitation

Limit of quantitation was calculated using following equation as per ICH guidelines. LOQ = 10 x SD/S

Where SD is the standard deviation of the Y- intercepts of the six calibration curves and S is mean slope of the five calibration curves.

3.7.7 Robustness

By introducing small changes in the mobile phase volume and duration of chamber saturation time, the effects on the results were examined. Mobile phase volume and duration of saturation were varied at 20±2ml (18, and 22 ml) and 20±10 min (10, and 30 min), respectively.

Robustness of the method was studied in triplicate at a concentration level of 400 ng/spot of Naproxen Sodium and 20ng/spot of Esomeprazole Magnesium Trihydrate.

3.7.8 ASSAY OF MARKETED FORMULATIONS

Twenty tablets of each formulation of Naproxen Sodium and Esomeprazole Magnesium Trihydrate were weighed and finely powdered. The tablet powder equivalent to 375/20 mg of Naproxen Sodium and Esomeprazole Magnesium Trihydrate was accurately weighed and transferred to a 100 ml volumetric flask, about 20 ml of methanol was added and the flask was sonicated for 15 min.

The volume was made up to 100 ml with methanol mixed well and filtered through Whatman paper no. 41. Pipette out 2.5 ml and dilute upto 25 ml with methanol. From the filtrate solution, 2µl was applied on the TLC plate and analysed as per the developed method.

IV. RESULTS AND DISCUSSION

There are numerous spectroscopic and chromatographic methods reported for assay of Naproxen Sodium and Esomeprazole Magnesium Trihydrate, but HPTLC method of analysis has not been reported so far and so the aim was to develop and validate HPTLC method of analysis for Naproxen Sodium and Esomeprazole Magnesium Trihydrate.

A suitable solvent system for the composition of the mobile phase for development of chromatogram was optimized by testing different solvent mixtures of varying polarity. Various mobile phases were evaluated. Mobile phase of Ethyl acetate: toluene: methanol (4:5.5:0.5 v/v/v) shows good resolution of both drugs. Chromatographic scanning of all tracks were done on 285 nm with R_f value at 0.73 ± 0.01 and 0.43 ± 0.01 respectively.

4.1 Linearity

Table: 2 Linearity of Naproxen Sodium (400-2400) and Esomeprazole Magnesium Trihydrate (20-120).

Concentration (ng/spot)	Peak Area (Mean ± S.D.); (n=3)	Concentration (ng/spot)	Peak Area (Mean ± S.D.); (n=3)
400	1438.1 ± 24.65	20	540.5 ± 12.66
800	3289.0 ± 34.92	40	1206.3 ± 32.84
1200	5506.2 ± 31.37	60	1971.2 ± 29.51
1600	7565.3 ± 51.48	80	2637.3 ± 52.47
2000	9528.4 ± 60.83	100	3215.6 ± 59.81
2400	11669.3 ± 60.15	120	3909.2 ± 52.76

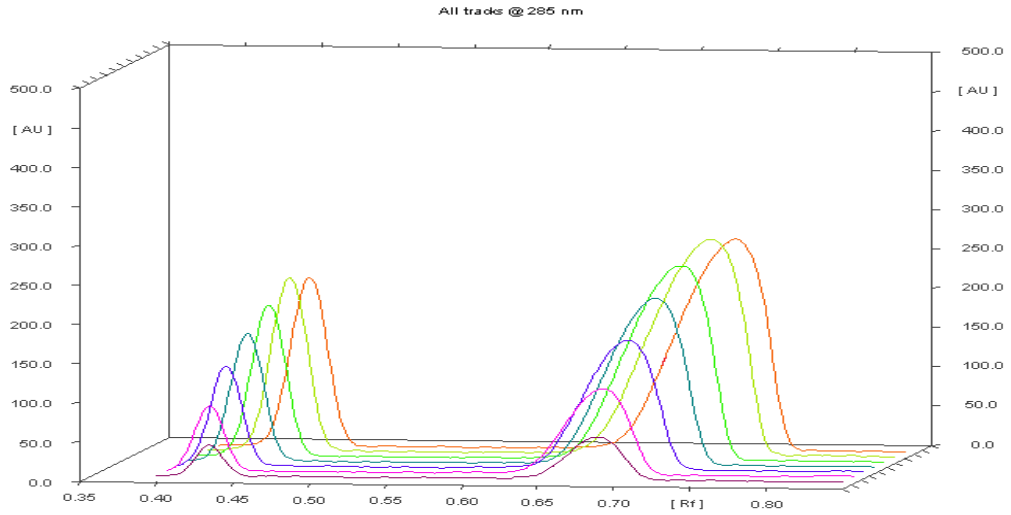

Fig.1 Overlain 3D Chromatogram shown in linearity of Esomeprazole Magnesium Trihydrate (20-120) and Naproxen Sodium (400-2400)

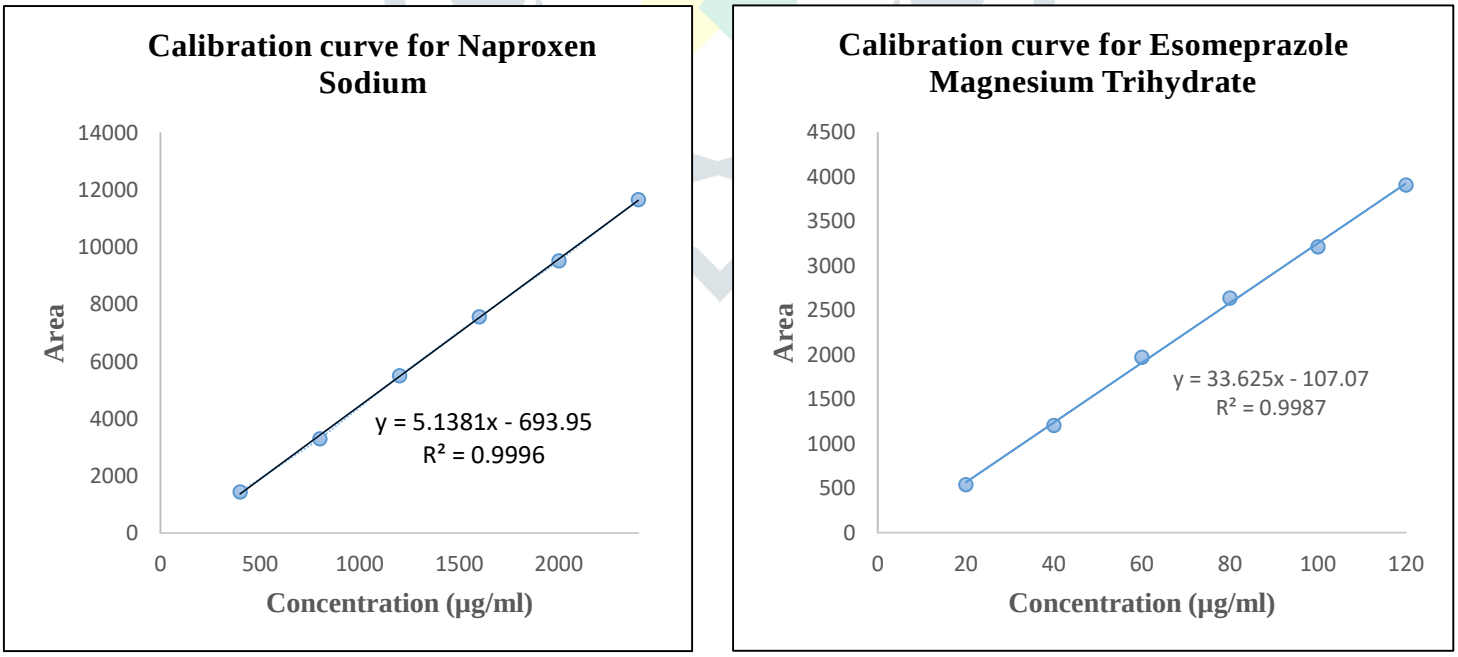

Fig.2 Calibration Curve of Naproxen Sodium (400-2400) and Esomeprazole Magnesium Trihydrate (20-120).

4.2 Precision

4.2.1 Repeatability of measurement and sample application

Table: 3 Repeatability of measurement and sample application Naproxen Sodium and Esomeprazole Magnesium Trihydrate.

Naproxen Sodium					Esomeprazole Magnesium Trihydrate				
Con.(µg/ml)	**Area**	**Mean**	**S.D**	**%RSD**	**Con.(µg/ml)**	**Area**	**Mean**	**S.D**	**%RSD**
400	1439.2	1434.883	4.72	0.3288	20	542.6	540.85	0.89	0.1649
400	1428.7				20	540.9			
400	1438.1				20	540.5			
400	1438.4				20	540.2			
400	1429.3				20	540.6			
400	1435.6				20	540.3			

4.2.2 Repeatability of sample application

Table: 4 Repeatability of sample application Naproxen Sodium and Esomeprazole Magnesium Trihydrate

Naproxen Sodium					Esomeprazole Magnesium Trihydrate				
Con.(µg/ml)	**Area**	**Mean**	**S.D**	**%RSD**	**Con.(µg/ml)**	**Area**	**Mean**	**S.D**	**%RSD**
400	1439.2	1436.22	6.63	0.4615	20	542.6	541.9	5.25	0.9681
400	1425.6				20	539.2			
400	1432.1				20	534.1			
400	1442.5				20	545.1			
400	1435.3				20	540.9			
400	1442.6				20	549.5			

4.2.3 Intermediate Precision

Table: 5 Intermediate Precision of Naproxen Sodium and Esomeprazole Magnesium Trihydrate

Naproxen Sodium					Esomeprazole Magnesium Trihydrate				
Concentration (ng/spot)	**Intraday precision**		**Interday precision**		**Concentration (ng/spot)**	**Intraday precision**		**Interday precision**	
60	5512.6	0.4542	1200	0.3170	1200	1971.6	0.6497	1963.1	0.5310
80	7542.6	0.3302	1600	0.4611	1600	2673.1	0.7450	2654.3	0.9265
100	9528.4	0.1398	2000	0.2696	2000	3241.3	0.5299	3259.1	0.7103

4.3 Recovery

Table: 6 Result of Recovery study for Naproxen Sodium

Level	Amount sample	Amount STD spiked	Total amount (mg)	Total area (AU) (Mean)	Recovered amount (mg) ± S.D. (n=3)	% recovery of spiked amount ± S.D. (n=3)
0%	375	-	375	1438.4	-	-
80%	375	300	675	2965.9	297.30 ± 0.65	99.1 ± 0.22
100%	375	375	750	3359.4	373.9 ± 2.27	99.7 ± 0.60
120%	375	450	825	3746.9	449.3 ± 3.98	99.85 ± 0.88

Table: 7 Result of Recovery study for Esomeprazole Magnesium Trihydrate

Level	Amount sample	Amount STD spiked	Total amount (mg)	Total area (AU) (mean)	Recovered amount (mg) ± S.D. (n=3)	% recovery of spiked amount ± S.D. (n=3)
0%	20	-	20	542.8	-	-
80%	20	16	36	1082.2	35.37 ± 0.08	100.27 ±0.83
100%	20	20	40	1213.9	39.28 ± 0.16	99.78 ± 0.67
120%	20	24	44	1347.2	43.24 ± 0.07	99.67 ± 0.48

4.4 LOD and LOQ

Table: 8 Result of LOD and LOQ

Parameter	Naproxen sodium	Esomeprazole Magnesium Trihydrate
Mean Y- intercept ± S.D.(n=3)	6499.38 ± 3845.74	2246.68 ± 1258.94
Mean slope ± S.D.(n=3)	4.73	3.23
LOD = 3.3 × (SD/Slope) (ng/spot)	2678.72	126.0
LOQ = 10 × (SD/Slope) (ng/spot)	8117.32	388.68

4.5 Robustness

Table: 9 Robustness

Sr. no	Parameter		% RSD Peak Area Naproxen Sodium	% RSD Peak Area Esomeprazole Magnesium Trihydrate
1	Mobile phase volume 20 ± 2	18	1.48	0.88
		22	0.99	0.63
2	Mobile Phase saturation 20 ± 10	10	0.84	1.15
		30	0.78	0.97

4.6 Assay of tablet formulation

Table: 10 Assay of tablet formulation

Brand Name	Label Claim	Naproxen Sodium		Esomeprazole Magnesium Trihydrate	
		Amount Found	% Label claim Obtained ±SD (n=3)	Amount Found	% Label claim Obtained ±SD (n=3)
ESPRA XN	375/20	375 ± 0.92	99.10 ± 0.22	20 ± 0.60	99.786 ± 0.67

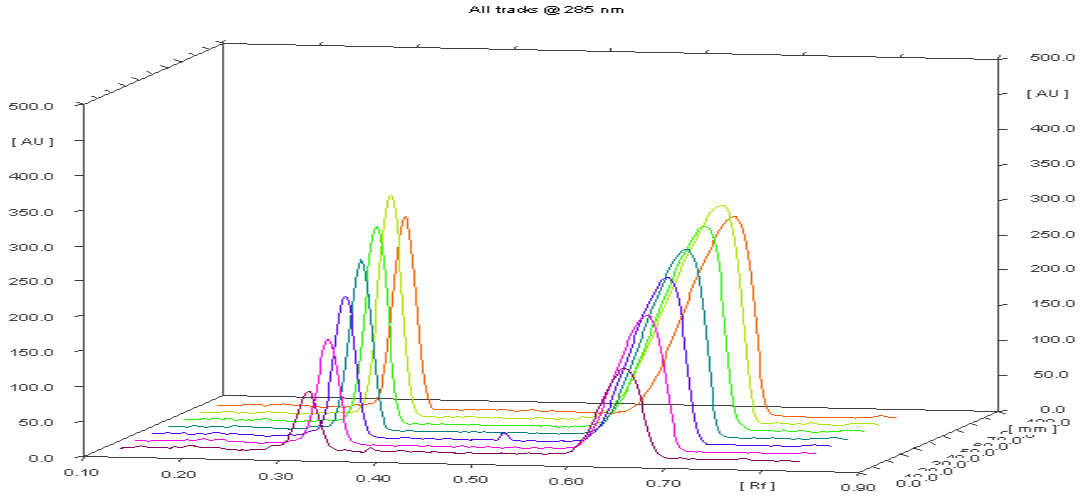

Fig.3 3 D Overlain of Linearity with Assay formulation.

V. CONCLUSION

Simple, precise and accurate method for the determination of Naproxen Sodium and Esomeprazole Magnesium Trihydrate in a pharmaceutical formulation.

VI. ACKNOWLEDGMENT

Special thanks to Mr. Devandrasinh Parmar, and Mr. Kaushik Patel

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