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Formulation and Evaluation of Controlled Release Roxatidine Acetate HCl Mucoadhesive Microspheres: *In-vivo* Study.

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ABSTRACT

Present study aims to prepare and evaluate Roxatidine acetate HCl mucoadhesive microspheres by ionotropic gelation method. Among all the formulations, M13 was selected as optimized formulation for mucoadhesive microspheres based on the evaluation parameters and drug release studies. In vitro release study of formulation M13 showed 99.4% in 12 h in a controlled manner, which is essential for disease like peptic ulcer. The release order kinetics for M13 was best fit with the highest correlation coefficient was observed in Higuchi model, indicating diffusion controlled principle. The innovator Rotane 150 mg conventional tablet showed the drug release of 96.45% within 1 h. In vivo studies revealed that the optimized formulation M13 gave the highest AUC and Tmax. The controlled release of drug from M13 also provides for higher plasma drug content and improved bioavailability.

Keywords: Roxatidine, mucoadhesiveness, in vivo bioavailability studies, microspheres.

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INTRODUCTION

Controlled drug delivery systems have acquired a centre stage in the area of pharmaceutical R & D sector [1]. The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body promptly and then maintain the desired drug concentration in the body over an entire period of treatment. This is possible through administration of conventional dosage form in a particular dose and particular frequency to provide a prompt release of drug. Therefore to achieve and maintain the concentration within the therapeutically effective range needs repeated administration in a day. This results in a significant fluctuation in a plasma drug level, leads to several undesirable toxic effects and poor patient compliance [2]. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems. The success of these microspheres is limited due to the short residence time at the site of absorption. It would therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres [3, 4].

Microsphere carrier systems, made from natural polymers are attracting considerable attention for several years, for sustained drug delivery. Today, those dosage forms which can control the release rates and which are target specific have a great impact in development of novel drug delivery systems. Microspheres are part of such novel delivery systems [5, 6].

The term microsphere is defined as a spherical particle with size from 1 µm to 1000 µm. The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 200 micrometer [7]. Microspheres are one of the multiparticulate drug delivery systems and are prepared by ionotropic gelation method by dropping drug loaded polymeric solution using syringe into the aqueous solution of polyvalent cations to obtain prolonged (or) controlled drug delivery to improve bioavailability or stability and to target drug to specific sites [8].

Mucoadhesive microspheres: The success of normal microspheres is limited due to short residence time at the site of absorption. Therefore, it would be advantageous to provide an intimate contact of the drug delivery systems with the absorbing membranes. This can be achieved by coupling bioadhesion characteristics to microspheres and formulating bioadhesive microspheres. These microspheres provide advantages such as efficient absorption and increased bioavailability of drugs owing to high surface-to-volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site [9, 10, 11].

Peptic ulcer disease, also known as a peptic ulcer or stomach ulcer, is a break in the lining of the stomach, first part of the small intestine or occasionally the lower esophagus. An ulcer in the stomach is known as a gastric ulcer while that in the first part of the intestine is known as a duodenal ulcer. The most common symptoms are waking at night with upper abdominal pain or upper abdominal pain that improves with eating. Common causes include the bacteria, *Helicobacter pylori* [12]**.**

Roxatidine acetate is a specific and competitive histamine H_2 receptor antagonist, which is used to treat gastric ulcers, Zollinger–Ellison syndrome, erosive esophagitis, gastro-oesophageal reflux disease and gastritis. Roxatidine has less bioavailability (80%) and lesser half life of 5 h^9. The aim of present work is to design and evaluate microspheres of Roxatidine acetate HCl *in vitro* to enhance its bioavailability and prolong residence time in stomach.

MATERIALS AND METHODS

Materials:

Cimetidine pure drug was generous gift from Splendid Laboratories, Pune, India. Sodium alginate was obtained from Pruthvi Chemicals, Mumbai. Chitosan, xanthan gum, kondagogu gum and sodium CMC were gifted from MSN Labs Ltd., Hyderabad. All other chemicals used were of analytical grade.

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Mucoadhesive microspheres:

Formulation of Roxatidine acetate HCl mucoadhesive microspheres

Roxatidine mucoadhesive microspheres were prepared using different polymers like sodium alginate, calcium chloride, chitosan, sodium cmc, xanthan gum, gum olibanum, guar gum and gum kondagogu by ionotropic gelation method.

Table 1: Formulation trials for Roxatidine mucoadhesive microspheres

Formulation code	Roxatidine acetate HCl (mg)	Sodium alginate	Sodium CMC(mg)	Calcium chloride	Xanthan gum	Gum olibanum
M1	1500	1 %	100	7%	1%	0.5%
M2	1500	1.2 %	150	7%	1.2%	0.5%
M3	1500	1.4%	200	7%	1.4%	0.5%
M4	1500	1.6%	250	7%	1.6%	0.5%
M5	1500	1.8%	300	7%	1.8%	0.5%
M6	1500	2%	350	7%	2%	0.5%
M7	1500	2.2%	400	7%	2.2%	0.5%
Formulation code	**Roxatidine acetate HCl (mg)**	**Sodium alginate**	**Chitosan (mg)**	**Calcium chloride**	**Guar gum**	**Gum kondagogu**
M8	1500	1%	10	10%	1%	0.5%
M9	1500	1.2%	15	10%	1.2%	0.5%
M10	1500	1.4%	20	10%	1.4%	0.5%
M11	1500	1.6%	25	10%	1.6%	0.5%
M12	1500	1.8%	30	10%	1.8%	0.5%
M13	1500	2%	35	10%	2%	0.5%
M14	1500	2.2%	40	10%	2.2%	0.5%

Procedure for the preparation of Roxatidine mucoadhesive microspheres:

The Roxatidine mucoadhesive microspheres were prepared by using ionotropic gelation technique. In this method, weighed quantity of Roxatidine acetate HCl was added to 100 ml sodium alginate, sodium CMC solution and other polymers, thoroughly mixed at 500 rpm. Resultant solution was extruded drop wise with the help of syringe and needle into 100 ml aqueous calcium chloride solution and stirred at 100 rpm. After stirring for 30 min the obtained microspheres were washed with water and dried at 60^{o}C for 4 h in a hot air oven and stored in desiccator.

Evaluation studies of Roxatidine acetate HCl mucoadhesive microspheres:

Micromeretic properties like particle size, angle of repose, bulk density, tapped density, compressibility index, Hausner's ratio and evaluation parameters like swelling index, drug entrapment efficiency, % yield and drug release order kinetics were performed and published by **Arifa Begum SK *et al*., 2016 [13].**

Mucoadhesive study:

The *in vitro* mucoadhesive test was carried out using small intestine from chicken. The small intestinal tissue was excised and flushed with saline. Five centimeter segments of jejunum were averted using a glass rod. Ligature was placed at both ends of the segment. 100 microspheres were scattered uniformly on the averted sac from the position of 2 cm above. Then the sacs were suspended in a 50 ml tube containing 40 ml of saline by the wire, to immerse in the saline completely. The sacs were incubated at 37^{0}C and agitated horizontally. The sacs were taken out of the medium after immersion for 1, 2, 3, 4, 5, 6, 7 and 8 h, immediately

repositioned as before in a similar tube containing 40 ml of fresh saline and unbound microspheres were counted. The adhering percent was presented by the following equation[9].

Mucoadhesion= (No. of microspheres adhered/ No. of microspheres applied) X 100

***In vitro* drug release studies:**

In vitro drug release studies for developed Roxatidine acetate HCl microspheres were carried out by using dissolution apparatus II paddle type (Electrolab TDL-08L). The drug release profile was studied in 900 ml of 0.1 N HCl at 37±0.5^{0}C temperature at 100 rpm. The amount of drug release was determined at different time intervals of 0, 1, 2, 3, 4, 6, 8, 10 & 12 h by UV-visible spectrophotometer (Shimadzu UV 1800) at 280 nm[10].

Drug excipient compatibility studies:

The drug excipient compatibility studies were carried out by Fourier transmission infrared spectroscopy (FTIR) method, Differential Scanning Calorimetry (DSC), SEM and release order kinetics along with stability studies were published by **Arifa Begum SK *et al*., 2016 [13]**.

***In-vivo* bioavailability studies:**

Animal Preparation:

Twelve New Zealand white rabbits of either sex were (weighing 2-3 kg) selected for this study, all the animals were healthy during the period of the experiment. Animals were maintained at room temperature 25^{0}C, RH 45% and 12 h alternate light and dark cycle with 100% fresh air exchange in animal rooms, uninterrupted power and water supply and rabbits were fed with standard diet and water ad libitum. The protocol of animal study was approved by the institutional animal ethics committee with IAEC No: 37/VCP/IAEC/2015/9/DBP/AE12/Rabbits.

***In vivo* Study design:**

Rabbits were randomly divided into two groups, each group contained six animals. The group A rabbits were fed with Roxatidine Mucoadhesive microspheres (optimized formulation M13), group B fed with Innovator product with equivalent dose to animal body weight. Blood samples (approximately 0.5 ml) were obtained with syringes by marginal ear vein at 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 16, 20 and 24 h post dose. During collection, blood sample has been mixed thoroughly with heparin in order to prevent blood clotting. Plasma was separated by centrifugation of the blood at 5000 rpm in cooling centrifuge for 5 min and stored frozen at −20°C until analysis.

Preparation of Plasma Samples for HPLC Analysis:

Rabbit plasma (0.5 ml) samples were prepared for chromatography by precipitating proteins with 2.5 ml of ice-cold absolute ethanol for each 0.5 ml of plasma. After centrifugation the ethanol was transferred into a clean tube. The precipitate was re suspended with 1 ml of acetonitrile by vortexing for 1 min. After centrifugation (5000 – 6000 rpm for 10 min), the acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a steam of nitrogen at room temperature. Samples were reconstituted in 200 µl of 70% of acetonitrile and 30% water was injected for HPLC analysis.

Determination of Roxatidine in Rabbit plasma by HPLC method:

Determination of Roxatidine using internal standard ranitidine by high performance liquid chromatography with a RP-C18 chromatographic column, Phenomenex Kinetex (150 mm × 4.6 mm i.d) and a mobile phase consisting of 20 mM KH_2PO_4 (pH 7.0) and acetonitrile (5:1, v/v at a flow rate 0.8 ml/min and the wavelength detection was 198 nm.

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Pharmacokinetic Analysis:

The pharmacokinetic parameters, peak plasma concentrations (C_{max}) and time to reach peak concentration (t_{max}) were directly obtained from concentration time data. In the present study, AUC_{0-t} refers to the AUC from 0 to 24 h, which was determined by linear trapezoidal rule and $AUC_{0-\alpha}$ refers to the AUC from time at zero hours to infinity.

The $AUC_{0-\alpha}$ was calculated using the formula $AUC_{0-t} + [C_{last}/K]$ where C_{last} is the concentration in μg/ml at the last time point and K is the elimination rate constant.

Various pharmacokinetic parameters like area under the curve [AUC], elimination half life ($t_{½}$). Volume of distribution (V_d), total clearance (Cl_T) and mean residence time for each subject using a non compartmental pharmacokinetic program. The pharmacokinetic parameters were performed by a non compartmental analysis using Win Nonlin 3.3® pharmacokinetic software (Pharsight Mountain View, CA USA). All values are expressed as the mean ± SD. Statistical analysis was performed with Graph Pad InStat software (version 3.00, Graph Pad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Difference with $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Mucoadhesive microspheres

Figure 1: Roxatidine acetate HCl Mucoadhesive microspheres Mucoadhesion study:

A **B**

Figure 2: Pictorial diagram showing mucoadhesive property of mucoadhesive microspheres in Chic Intestine at 0 min (A) & after 8 hr (B)

All fourteen formulations were evaluated for various micromeretic and physicochemical parameters and found to be within the limits**.** Among all the formulations, M13 shown best results of particle size, bulk density, tapped density, angle of repose and Carr's index. The percentage yield and entrapment efficiency of all the formulations were measured by assay method and found to be within the limits. The formulation M13 showed good percentage yield and entrapment efficiency, swelling index and mucoadhesiveness.

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***In vitro* drug release studies:**

Roxatidine acetate HCl microspheres were evaluated for *in vitro* drug release studies in 0.1N HCl and the results were depicted in **Table 2**. The formulation M13 showed best drug release of 99.4% within 12 h. The drug release of optimized formulation M13 was in controlled manner when compared with innovator product Rotane i.e., 96.45% within 1 h.

Table 2: *In-vitro* cumulative % drug release of Roxatidine acetate HCl Mucoadhesive microspheres Formulations

Time (h)	M1	M2	M3	M4	M5	M6	M7	Innovator (Rotane 150 mg)
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	18.21±0.32	16.51±0.11	16.51±0.22	15.26±0.23	15.19±0.11	14.09±0.16	14.09±0.22	96.45±0,12
2	39.32±0.15	33.62±0.21	35.32±0.11	33.67±0.15	29.02±0.16	26.33±0.43	26.33±0.24	---
4	50.21±0.11	50.02±0.31	51.73±0.65	48.07±0.11	45.31±0.13	35.75±0.88	35.75±0.15	----
6	64.46±0.16	67.63±0.22	66.72±0.43	60.96±0.16	55.43±0.12	55.06±0.76	55.06±0.17	----
8	81.08±0.32	83.47±0.32	75.23±0.16	79.28±0.21	71.98±0.21	73.53±0.54	73.53±0.54	----
10	88.39±0.16	90.36±0.17	85.31±0.32	93.27±0.33	88.53±0.11	80.42±0.34	80.42±0.55	----
12	91.27±0.99	93.44±0.77	91.82±0.22	90.74±0.17	93.22±0.16	91.14±0.21	87.14±0.76	----

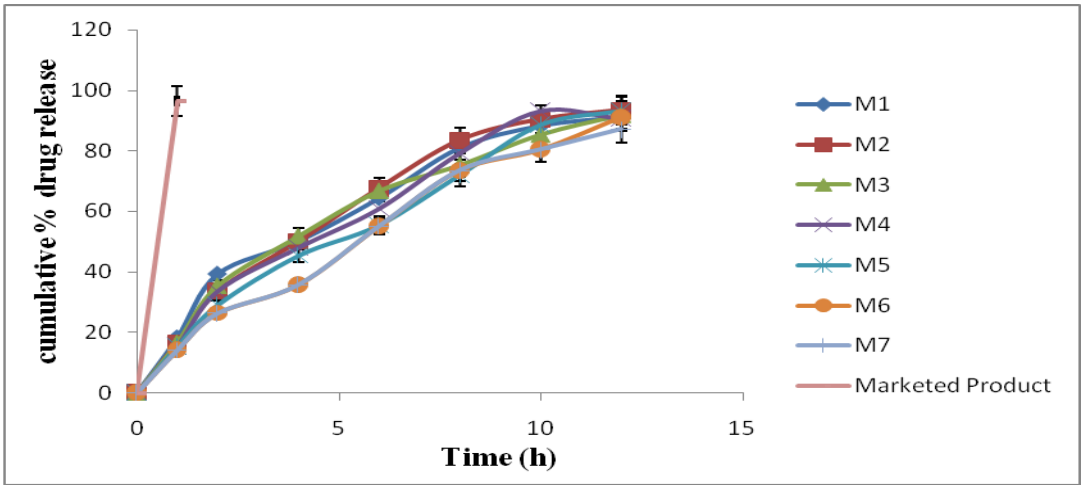

Figure 3: *In-vitro* cumulative % drug release of Roxatidine acetate HCl Mucoadhesive microspheres formulations

Table 3: *In-vitro* cumulative % drug release of Roxatidine acetate HCl mucoadhesive microspheres formulation

Time (h)	M8	M9	M10	M11	M12	M13	M14
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	10.21±0.66	8.96±0.11	10.83±0.56	6.51±0.22	7.63±0.22	11.23±0.22	8.21±0.11
2	17.7±0.32	16.05±0.15	19.22±0.66	14.33±0.15	17.44±0.21	24.91±0.18	18.82±0.21
4	28.52±0.55	26.56±0.16	27.83±0.98	21.57±0.22	24.89±0.15	33.51±0.87	29.64±0.22
6	40.71±0.32	38.45±0.17	36.54±0.43	30.08±0.32	37.97±0.16	43.52±0.98	45.75±0.32
8	56.54±0.22	52.36±0.26	49.86±0.32	42.72±0.11	49.86±0.12	60.94±0.87	54.96±0.16
10	70.66±0.34	72.04±0.12	61.37±0.11	59.23±0.43	60.64±0.32	69.48±0.16	66.18±0.17
12	88.43±0.45	88.55±0.32	83.45±0.32	78.74±0.22	72.17±0.21	99.4±0.22	79.03±0.42

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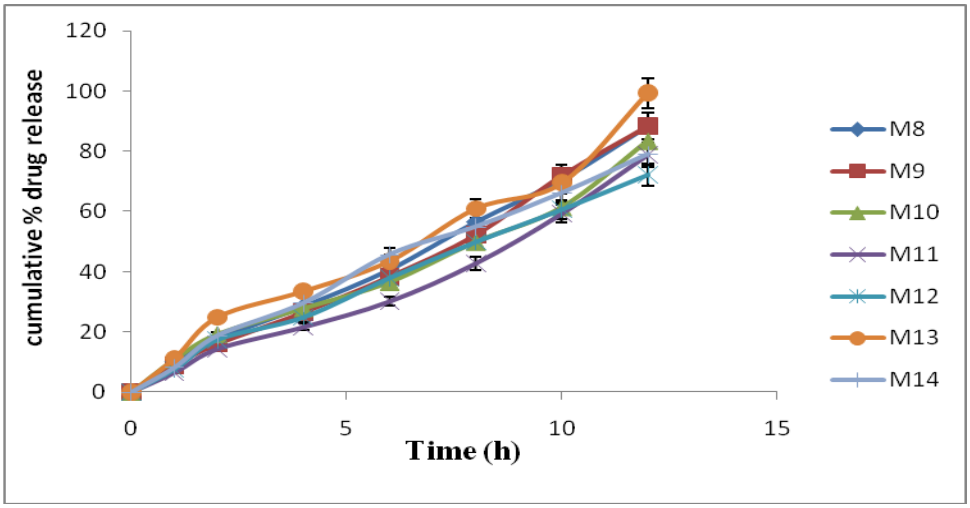

Figure 4: *In-vitro* cumulative % drug release of Roxatidine acetate HCl mucoadhesive microspheres formulation

***In vivo* bioavailability studies:**

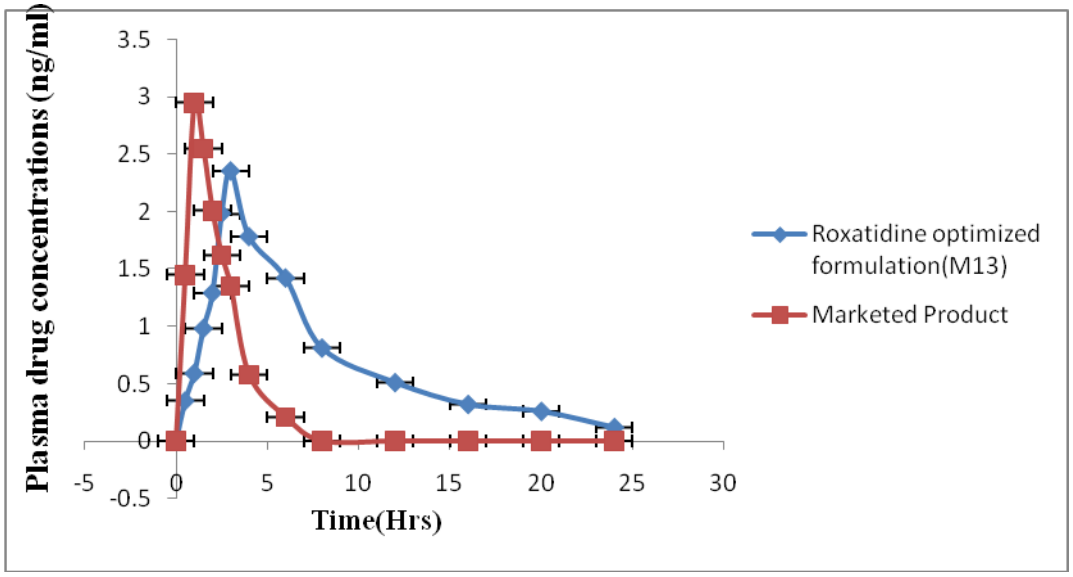

Figure 5: Plasma concentrations at different time intervals for Roxatidine optimized formulation (M13) and Marketed Product

Table 4: Comparison of pharmacokinetic parameters of Roxatidine optimized formulation and Marketed Product

Parameters	Roxatidine Optimized formulation	Marketed Product
C_{max} (ng/ml)	2.35±0.01	2.95±0.01
AUC_{0-t}(ng hr/ml)	15.15±1.12	8.21±1.26
$AUC_{0-\infty}$ (ng hr/ml)	19.42±1.16	11.15±1.13
T_{max} (hr)	3.00±0.05	1.00±0.04
$t_{1/2}$ (hr)	5.85 ± 0.41	3.91 ± 0.01
Kel (hr^{-1})	1.93 ± 0.11	1.15 ± 0.33

Bioavailability parameters:

Mean plasma concentration profiles of prepared Roxatidine optimized formulation and marketed product were presented in **Figure 5**. Roxatidine optimized formulation exhibited as sustained release *in vivo* when compared with innovator tablet. All the pharmacokinetics parameters displayed in **Table 4**. Roxatidine

marketed drug was available in plasma within an hour after its oral administration. The T_{max} of the test Roxatidine was significantly different ($p < 0.05$) from that of the standard. Low T_{max} value for the reference drug (1.00±0.04 h) indicates rapid absorption while the higher T_{max} of the test drug (3.00±0.05 h) suggests slower absorption. This delayed absorption of test preparation is most likely due to the sustained release of the drug. In order to estimate the amount of drug absorbed from the test formulation, the relative bioavailability was calculated from the AUC of the reference and test formulations (8.21±1.26 ng. hr/ml for the reference product versus. 15.15±1.12 ng. hr/ml for the test formulation). The results indicated that the test formulation could increase the bioavailability of Roxatidine in rabbits effectively. In this study, the Roxatidine test formulation produce higher bioavailability than that of a marketed product.

CONCLUSION

Mucoadhesive microspheres of Roxatidine acetate HCl were formulated by ionic gelation method, using different polymers like sodium alginate, chitosan and calcium chloride in different concentrations with the formulation code M1-M14 were prepared. All the formulations were evaluated for their various micromeretic and physicochemical parameters and found to be within the limits.

All the 14 formulations of mucoadhesive microspheres were exposed to mucoadhesion test. The formulation M13 showed the high percentage of mucoadhesive property and 95% of adhesion nature. The cumulative % drug release of the formulation M13 was found to be 99.4% within 12 h. *In vivo* evaluation carried out using for the optimized formulation which showed controlled release for 12 h and more bioavailability when compared with reference standard. The developed microspheres are safe and are the need of pharmaceutical industry as an alternate for effective management of ulcer disease. These results may be due to the prolongation of the contact time of the microspheres to the mucin which increases the duration of the action of the drug and its bioavailability.

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