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FORMULATION DEVELOPMENT AND IN-VITRO EVALUATION OF ANTI VIRAL DRUG GASTRORETENTIVE MICROSPHERES

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ABSTRACT

The present work was the Formulation Development and In-Vitro Evaluation Of lopinavir Gastroretentive Microspheres. Different polymers like sodium alginate, sodium carboxy methyl cellulose, HPMC K4M, HPMC K100M were utilized in the trials. All the physical evaluations are carried in preformulation studies were carried out on all the three different polymers utililized. All the formulations exhibited values within the acceptable range. Microspheres were evaluated for buoyancy studies, drug entrapment efficient. Release studies were carried out in 0.1N HCL for 12 hours. Evaluated samples for all the four polymer system. Results indicated that formulation F7, gave 90.12 % release up to 12 hrs which is formulated with HPMC K100M and HPMCK4M combination. Assay was carried out for formulation F7 and was found to be 88.69 %. The mechanism of drug release from microspheres follows Non-Ficknian release. Remaining formulations gave fluctuating release profiles. The formulation F7 was considered to be better among the trails accomplished.

KEYWORDS: Gastroretentive Microspheres, buoyancy studies, drug entrapment efficient, kinetic studies.

INTRODUCTION

During the last three decade many studies have been performed concerning the sustained release dosage form of drugs, which have aimed at the prolongation of gastric emptying time (GET). The GET has been reported to be from 2 to 6 hours in humans in the fed state. Accordingly orally, sufficient bioavailability and prolongation of the effective plasma level occasionally cannot be obtained. Gastric emptying of dosage forms is an extremely variable process and ability to prolong and control the emptying time is a valuable asset for dosage forms, which reside in the stomach for a longer period of time than conventional dosage forms. Several difficulties are faced in designing controlled release systems for better absorption and enhanced bioavailability. One of such difficulties is the inability to confine the dosage form in the desired area of the gastrointestinal tract. Drug absorption from the gastrointestinal tract is a complex procedure and is subject to many variables. It is widely acknowledged that the extent of gastrointestinal tract drug absorption is related to contact time with the small intestinal mucosa. Thus, small intestinal transit time is an important parameter for drugs that are incompletely absorbed.[1]

Gastro retentive systems can remain in the gastric region for several hours and hence significantly prolong the gastric residence time of drugs. Prolonged gastric retention improves bioavailability, reduces drug waste, and improves solubility for drugs that are less soluble in a high pH environment. It has applications also for local drug delivery to the stomach and proximal small intestines. Gastro retention helps to provide better availability of new products with new therapeutic possibilities and substantial benefits for patients.[2]

It is evident from the recent scientific and patient literature that an increased interest in novel dosage forms that are retained in stomach for a prolonged and predictable period of time exists today in academic and industrial research groups. One of the most feasible approaches for achieving a prolonged and predictable drug delivery in the GI tract is to control the gastric residence time (GRT), i.e. gastro retentive dosage form (GRDF or GRDS). GRDFs extend significantly the period of time over which the drugs may be released. They not only prolong dosing intervals, but also increase patient compliance beyond the level of existing controlled release dosage form. Dosage form with prolonged GRT, i.e. gastro retentive dosage form (GRDF), will bring about new and important therapeutic options such as – This application is especially effective in sparingly soluble and insoluble drugs. It is known that, as the solubility of a drug decreases, the time available for drug dissolution becomes less adequate and thus the transit time becomes a significant factor affecting drug

absorption. To override this problem, erodible, gastro retentive dosage forms have been developed that provide continuous, controlled administration of sparingly soluble drugs at the absorption site.

GRDFs greatly improve the pharmacotherapy of the stomach through local drug release, leading to high drug concentration at the gastric mucosa. (For e.g. Eradicating Helicobacter pylori from the sub mucosal tissue of stomach) making it possible to treat stomach and duodenal ulcers, gastritis and oesophagitis, reduce the risk of gastric carcinoma and administer non- systemic controlled release antacid formulations (calcium carbonate).[3]

GRDFs can be used as carriers for drugs with so-called absorption windows. These substances for e.g. antiviral, antifungal and antibiotic agents (sulphonamides, quinolones, penicillin, cephalosporin, amino glycosides, tetracycline etc.), are taken up only from very specific sites of the GI mucosa.

Lopinavir is a dicarboxylic acid diamide that is amphetamine is substituted on nitrogen by a (2,6-dimethylphenoxy)acetyl group and on the carbon alpha- to nitrogen by a (1S,3S)-1-hydroxy-3-{[(2S)-3-methyl-2-(2-oxotetrahydropyrimidin-1-yl)butanoyl]amino}-4-phenylbutyl group. An antiretroviral of the protease inhibitor class, it is used against HIV infections as a fixed-dose combination with another protease inhibitor, ritonavir. It has a role as an antiviral drug, a HIV protease inhibitor and an anticoronaviral agent. It is a member of amphetamines and a dicarboxylic acid diamide.[4]

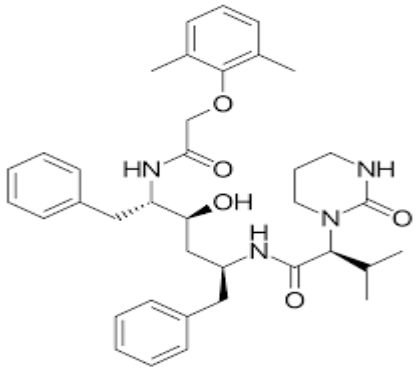

Figure 1: Chemical structure of Lopinavir.

Experimental Work

MATERIALS AND METHODS

Lopinavir gift sample from Molecules India Pvt Ltd, Chennai, Sodium alginate, Sodium carboxy methyl cellulose, Hydroxy propyl methyl cellulose K4M, Hydroxy propyl methyl cellulose, K100M Sodium phosphate dibasic dehydrate, Potassium dihydrogen ortho Phosphate, Sodium chloride, Paraffin liquid colorless light, Polysorbate 80 (tween 80), Dichloromethane, Methanol, n-hexane purchased from SD fine chemical Mumbai. All the instruments used in the work was calibrated.

METHODOLOGY[6]

PREFORMULATION

Preformulation testing is an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. It is the first step in the rational development of dosage forms.

Preformulation studies relate to pharmaceutical and analytical investigation carried out in supporting formulation development efforts of the dosage forms.

The following preformulation studies were performed for obtained sample of drug.

ORGANOLEPTIC PROPERTIES

Colour and nature

Taste and odour

PHYSICAL CHARACTERISTICS

Flow properties

Bulk density

Tapped density

Measurement of powder compressibility

Hausner ratio

Melting point

SOLUTION PROPERTIES

pH of the solution

Solubility

IDENTIFICATION OF DRUG AND COMPATABILITY STUDY

Drug –excipient compatibility studies

UV SPECTROSCOPIC METHOD FOR ANALYSIS OF LOPINAVIR [7-8]

Preparation of stock solution

Weighed accurately 100mg of drug and transferred it to 100ml volumetric flask, then add water and the volume was made up to 100ml with water**.**

Preparation of standard solution

Then pipette out 1ml from above solution in a 10ml volumetric flask and made the volume with water to 10ml. From standard stock solution 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1ml solution was pipette out in five 10ml volumetric flasks and make up the volume with water to get 2 µg/ml,4 µg/ml,6 µg/ml,8 µg/ml,10 µg/ml.

CALIBRATION CURVE OF LOPINAVIR

Measured the absorbance of the above prepared standard solution at 296 nm. plotted a graph of concentration (in µg/ml on X axis and absorbance (in nm) on Y axis

Table 11: Calibration curve for Lopinavir.

S.No.	Concentration(g/	Absorbance
1	0	0
2	2	0.152
3	4	0.279
4	6	0.392
5	8	0.514
6	10	0.623
Slope	0.0632	
r^2	0.9987	

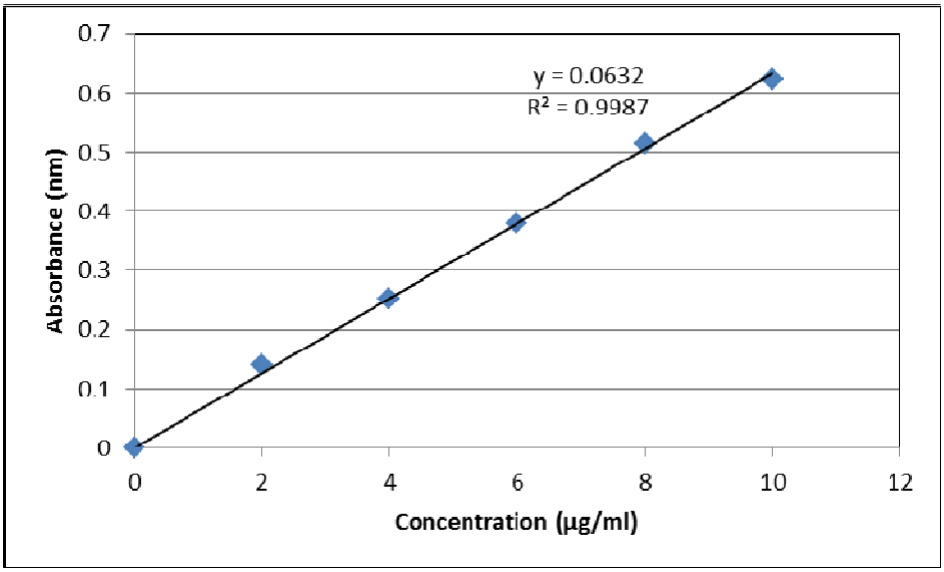

Fig.11: Calibration curve for Lopinavir.

FORMULATIONS OF LOPINAVIR FLOATING MICROSPHERES

Table 12: Formulations.

INGREDIENTS (mg)	FORMULATION BATCHES									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Lopinavir	200	200	200	200	200	200	200	200	200	200
HPMC K4M	100	-	-	-	500	500	500	-	-	-
Sodium alginate	-	100	-	-	500	-	-	500	500	-
Sodium CMC	-	-	100	-	-	500	-	500	-	500
HPMC K100M	-	-	-	100	-	-	500	-	500	500
Methanol	5	5	5	5	5	5	5	5	5	5
Dichloromethane	5	5	5	5	5	5	5	5	5	5

Floating microspheres were prepared by the solvent evaporation method using 1000 mg of Lopinavir and with different polymer as shown in Table, were dissolved in methanol (5 ml), dichloromethane (5 ml) with vigorous agitation to form uniform drug polymer dispersion. This was slowly poured into the dispersion medium consisting of light liquid paraffin (100ml) containing 1.1% tween 80. The system is stirred using propeller at 1000 rpm at room temperature for 1hr 30 min – 2 hr. The liquid paraffin was decanted and the microparticles were separated by filtration through a whatmann filter paper, was head thrice with 180 ml of n-Hexane and air dried for 6-8 hrs.

EVALUATION OF LOPINAVIR HCL FLOATING MICROSPHERES[9-10]

Micromeritic properties

Angle of repose

Determination of bulk density and tapped density

Percentage yield of microspheres

The prepared microspheres of all batches were accurately weighed. The weight quantity of prepared microspheres was divided by the total amount of all the excipients and drug used in the preparation of the microspheres, which give the total percentage yield of floating microspheres. It was calculated by using following equation,

$$\text{Percentage yield} = \frac{\text{Actual yield of product}}{\text{Total weight of excipients and drug}} \text{X } 100$$

Drug entrapment

According to dose 100mg actual drug present in total microspheres (drug 1gm+ excipients) is calculated. That amount mixed in 0.1 M HCL by sonication. From that pipette 1ml into 10ml volumetric flask and made upto 10ml using buffer. The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically at 296 nm against appropriate blank. The amount of drug entrapped in the microspheres was calculated by the following formulae.

$$\text{Percentage drug entrapment} = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \text{ X } 100$$

Buoyancy studies

Microspheres (300mg) were spread over the surface of a USP XXIV dissolution apparatus type II filled with 900 ml of 0.1 M HCL. The medium was agitated with a paddle rotating at 75 rpm for 12 h. The floating and the settled portions of microspheres were recovered separately. The microspheres were dried and weighed. Buoyancy percentage was calculated as the ratio of the mass of the microspheres that remained floating and the total mass of the microspheres.

***Invitro* dissolution studies Preparation of buffer solution**

Measure 4 ml of concentrated Hydrochloric acid, in a 1000ml volumetric flask and make up the volume upto 1000ml using distilled water and stir until it mix.

***In-vitro* release profile:**

Medium : 0.1 M HCL
Apparatus : USP II
(Paddle) Speed : 75 rpm
Time : 1hr, for every 2hrs up to 12hr Temperature : 37.5 C
max : 296 nm

Perform the test on microspheres place in each dissolution vessel containing 900ml of 0.1 M HCL maintained at 37 C ± 0.5 ˚C. At specified time withdrawn requiredamount the sample and replace the same amount with (maintain sink conditions) phosphate buffer, then absorbance was taken and calculate percentage release.

Kinetics of drug release[11]

The *invitro* dissolution profile of all batches were fitted to Zero order, First order,Higuchi model, Korsemeyer-peppas model to ascertain the kinetic modeling of drug release. Correlation coefficient (r^2) values were calculated for linear curves obtained by the regression analysis of the above plot.

RESULTS AND DISCUSSIONS

PRE-FORMULATION STUDIES

Organoleptic properties

These tests were performed as procedure given, Preformulation part.

The results are illustrated in following table.

Table: Organoleptic properties.

Test	Specifications/limits	Observations
Color	White to off white	Off White powder
Odour	odorless	odorless

The results complies as per specifications

Angle of repose

It was determined as per procedure preformulation in material and method part.

The results are illustrated in following table.

Material	Angle of repose
Lopinavir sulphate	$29^0.28$”

Bulk density and tapped density.

It was determined as per procedure given preformulation in material and method part. The results are illustrated in table.

Table: Density.

Materials	Bulk Density(gm/ml)	Tapped density(gm/ml)
Lopinavir sulphate	0.19	0.26

Powder compressibility

It was determined as per procedure given in preformulation in material and method part. The results are illustrated in table.

Table Powder compressibility

Material	Compressibility index	Hausner ratio
Lopinavir sulphate	28.04%	1.34

Melting point

It was determined as per procedure given in preformulation in material and method part. The results are illustrated in following table**.**

Material	Material point	Result
Lopinavir sulphate	165^{o}C	Complies

SOLUTION PROPERTIES

pH of the solution

It was determined as per procedure given in preformulation in material and method part. The results are illustrated in following table**.**

Table 19: pH.

Material	Test	Specification	Observation
Lopinavir sulphate	pH	7.5	7.5
The result complies as per specification			

Solubility

It was determined as per procedure given in preformulation in material and method part. The results are illustrated in following table.

Table Solubility.

Test	Specification	Result
solubility	Freely soluble in water, Sparingly soluble in DMSO, ethanol, methanol	Complied

DRUG-EXCIPIENT COMPATABILITY STUDIES

DISCUSSION

Drug excipient interactions play a vital role with respect to release of drug from formulation amongst others. FTIR techniques have been used here to study the physical and chemical interaction between drug and excipient used.

Table. No. 23: Evaluation of Microspheres.

Batch NO.	Angle of repose	Bulk density (g/ml)	Tapped desnity (g/ml)	Carr's index	Huasner ratio
F1	25.08	0.2826	0.3177	10.38	1.11
F2	23.52	0.2671	0.3242	14.07	1.19
F3	24.24	0.2841	0.3318	16.47	1.15
F4	27.62	0.2853	0.342	16.13	1.19
F5	24.09	0.2965	0.3446	14.47	1.16
F6	27.37	0.2924	0.3321	11.94	1.13
F7	26.64	0.2768	0.3394	13.68	1.22
F8	24.71	0.2891	0.3503	16.04	1.21
F9	26.14	0.2965	0.3446	13.54	1.16
F10	25.5	0.2721	0.3242	15.07	1.19

DISCUSSION

The angle of repose for the formulations F1-F10 was found to be in the range 23.52˚ to 27.62˚ shows good flow property Compressibility index for the formulations F1-F10 found between 10.38% to 16.13% indicating the good flow property.

Table 24: Percentage Yield, *Invitro* Buoyancy, Drug Entrapment Efficiency of Floating Microspheres Of Lopinavir Sulphate.

Batch no	Percentage yield	*Invitro buoyancy*	Entrapment efficiency
F1	84.52	85.65	82.6
F2	82.98	83.75	81.73
F3	83.54	84.82	82.17
F4	79.89	80.23	78.61
F5	85.15	86.24	83.04
F6	88.91	89.54	87.39
F7	90.21	91.66	88.69
F8	86.78	87.26	83.92
F9	85.59	86.92	83.47
F10	87.66	88.22	85.21

Percentage yield

The maximum percentage yield was found in F7 formulation and was noted to be 90.21 % among all formulations.

The floating microspheres were prepared with different and combination of two polymer of HPMC K4M, HPMC K100M, Sodium Alginate and Sodium CMC to investigate the influence of encapsulation efficiency and were used to determine its influence on floating behavior.

***Invitro* Buoyancy**

DISCUSSION

The different polymers with same ratios of formulation were selected for optimization of their buoyancy property. The formulations in which combination of HPMC K4M, HPMC K100Mare giving the better results.

The formulations, are selected as the best formulations depending upon their buoyancy, encapsulation efficiency. From the results of all the ten formulations, it is confirmed that the change in polymers of Sodium Alginate, Sodium CMC, HPMC K4M, K100M influences the properties of the formulations. The formulation F7 with drug and combination of two polymer 1:1 ratio, is giving the best result of buoyancy property.

The microspheres, having lower densities (having a hollow core) exhibited buoyancy and are expected to be retained in gastric environment for more than 12 hrs. This may be attributed to a decrease in density of microspheres with an increase in polymer concentration.

Entrapment efficiency

DISCUSSION

The percentage entrapment efficiency of various formulation parameters of the prepared microspheres were shown in table. The entrapment efficiency varied from 78.61 to 88.69.

The formulation F7 is having high encapsulation efficiency of 88.69% and F4 is having low encapsulation efficiency of 78.61%.

The low encapsulation is because of using single polymer of HPMC K100M than the drug concentration where the quantity of HPMC K100M is insufficient to entrap the drug. The high encapsulation efficiency is because of using combination of polymers of HPMC K100M, HPMC K4 where the increase in the HPMC concentration forms larger microspheres encapsulating more amount of drug.

Table 25: *Invitro* Release Profile.

Time (hours)	BATCH NO.									
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**
1	20.03	12.5	14.18	10.1	12.91	10.56	8.25	15.23	12.11	9.54
2	35.56	32.56	31.56	28.19	22.12	15.99	13.11	25.76	22.77	13.84
4	54.14	53.29	45.25	62.43	35.12	28.21	24.72	37.45	30.99	24.15
6	69.65	77.38	63.14	76.37	52.64	44.14	40.99	53.28	48.12	43.9
8	89.14	96.98	92.2	98.24	78.1	58.9	56.25	79.5	69.8	54.6
10	69.65	77	63.1	76.3	94.4	78.6	72.84	90.4	93.5	72.7
12	89.14	96	92	98.2	90.47	87	90.12	87	90.12	85.2

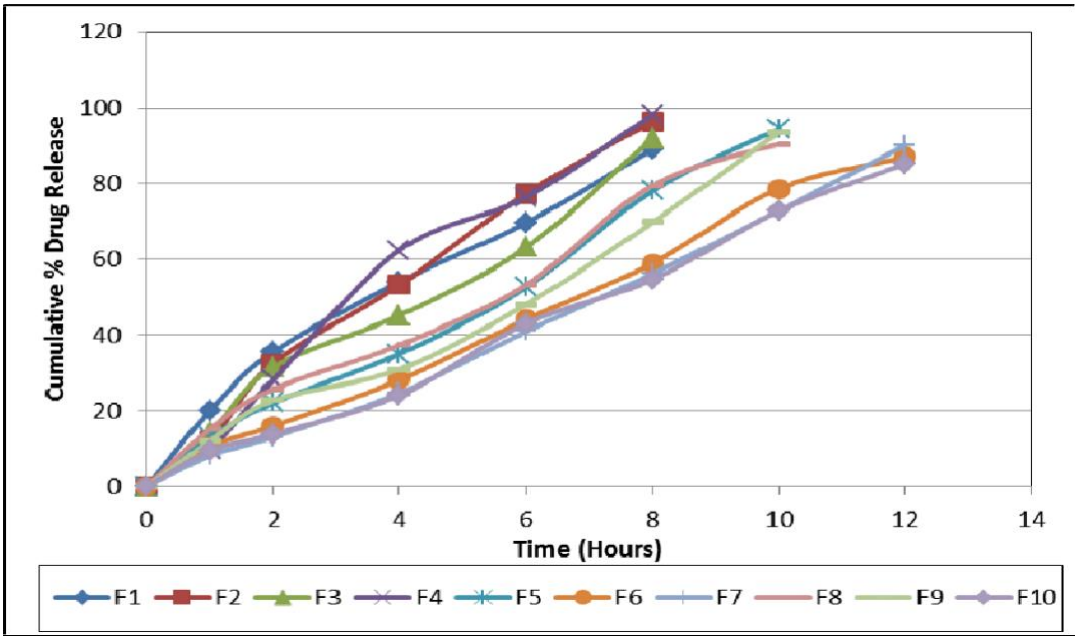

Fig. 16: *Invitro* Dissolution Release Profile for F1 – F10 Formulations.

Kinetics of drug release

Table 27: Regression Coefficient of F7.

Formulation	Regression coefficient (r2) values			
	Zero order	First order	Higuchi model	Korsemeyer -pepper
Lopinavir Floating Microsphere	0.9955	0.8278	0.8945	0.84

n = 0.9735

The regression coefficient values and n values show that the drug releases follow Non - Fickian release (Diffusion and swelling).

SUMMARY

The present study involves formulation and evaluation of sustained release floating microspheres of Lopinavir sulphate. Endeavours with respect to floating mechanism are inculcated in the formulation to achieve longer stay of microsphere in stomach which happen to better site of absorption for the selected drug.

Preformulation studies involving organoleptic bulk density, tapped density, angle of repose, compressibility of index, hausner ratio, melting point range, pH, solubility were carried out as per IP specification.

Drug excipient compatibilities were carried out and evaluation and FT-IR, SEM. This showed no significant change in any way to the Mixture.

Different polymers like sodium alginate, sodium carboxy methyl cellulose, HPMC K4M, HPMC K100M were utilized in the trials. All the physical evaluations are carried in preformulation studies were carried out on all the three different polymers utililized. All the formulations exhibited values within the acceptable range.

Microspheres were evaluated for buoyancy studies, drug entrapment efficient. Release studies were carried out in 0.1N HCL for 12 hours. Evaluated samples for all the four polymer system. Results indicated that formulation F7, gave 90.12 % release up to 12 hrs which is formulated with HPMC K100M and HPMCK4M combination. Assay was carried out for formulation F7 and was found to be 88.69 %. The mechanism of drug release from microspheres follows Non-Ficknian release.

Remaining formulations gave fluctuating release profiles. The formulation F7 was considered to be better among the trails accomplished.

CONCLUSION

The ultimate goal for sustained drug release is to maximize therapeutic activity while minimizing the negative side effects of the drug. In this regard, floating microspheres have emerged as a novel drug delivery system to treat HIV with Lopinavirsulphate. The type of polymer affects the drug release rate and the mechanism. Polymer swelling is crucial in determining the drug release rate and is also important for flotation. A lesser FLT and a prolonged floating duration could be achieved by using different polymer combinations. In this study sustained release Floating Microsphere approach for Lopinavirpurposes that with hydrophilic polymers the GI retention can be enhanced and reduce frequency of

dosing, thereby minimizing the occurrence of side effects, site specificity, increase the effectiveness of the drug and better patient compliance This gives a signal to extending this approach to similar combinations of drugs used in clinical practice so as to improve bioavailability of poorly absorbed drugs in GIT.When these floating microspheres compared to other floating dosage forms like floating tablets have bulk density less than gastric fluid and so remain buoyant in the stomach for prolonged period of time and these are used as multiunit dosage form and drug release optimization and show efficiency level. So, Sustained release floating microspheres of Lopinavirmay provide a convenient dosage form for achieving best performance and release and show good bioavailability.

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