Rapid Stability-indicating RRLC Method for Simultaneous Estimation of Irbesartan and its Related Impurities

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A green, novel gradient stability-indicating reverse phase rapid resolution liquid chromatographic method was developed and validated for simultaneous estimation of irbesartan and along with six related impurities in active pharmaceutical ingredient samples. The chromatographic separation was achieved on Kromasil C8 (3.5 μm, 150×4.6 mm) short column with 0.1% v/v ortho-phosphoric acid and acetonitrile as mobile phase using gradient elution. The developed method showed good resolution between irbesartan and its six related impurities and were eluted within 15 min. run time of LC chromatogram. Regression analyses indicate correlation coefficient value greater than 0.999 for irbesartan and its six related impurities. The limit of detection for irbesartan and the known related impurities were observed at a level below 0.004% (0.019 μg/ml and the method is showing better recoveries for irbesartan (99.6–100.7%) and also for its six known impurities (88.5–98.9%). The test solution and related substances were found to be stable in the diluents for 24 h. The developed stability-indicating method is found to be rapid, accurate, precise, linear, specific, sensitive, rugged, robust, and stability-indicating. The application of developed method was also verified by an assay of irbesartan and related substances in commercial bulk drug samples and more essentially, the method is economic and environment friendly than the other published methods.

Key words: Irbesartan RRLC, stability-indicating, related substances, validation

Irbesartan (IRB) is chemically described as 2-butyl-3-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]-1,3-diazaspiro[4.4]non-l-en-4-one. Its empirical formula is $C_{25}H_{28}N_6O$, and molecular weight is 428.5 amu. IRB is an active non-peptide specific angiotensin II receptor antagonist (AT1 subtype) used as anti-hypertensive agent. Hypertension is the most prevalent cardiovascular disease in the developed as well as developing countries, affecting as many as one quarter of the adult population.

Furthermore, hypertension is an independent risk factor for cardiovascular diseases and associated with an increased incidence of stroke and coronary heart disease. Angiotensin II antagonists are major drugs used in hypertension management in the recent decade. Their lower side effect profile and specificity in the action provided a good condition for patient compliance as well as effectiveness. Therefore, these drugs are used as first line treatment for hypertension[1-4]. Stability testing of new drug substances and drug products requires a stress testing, which should be carried out to elucidate the inherent stability characteristics of the active substance. It suggests that the degradation products, which were formed under variety of conditions, should be identified and degradation pathways are to be established[5]. The literature survey reveals that several methods[6-13] were reported for the determination of IRB and hydrochlorothiazide. The methods[14-17] for IRB in combination with other drugs in plasma and serum were done by high-performance liquid chromatography (HPLC) and the few analytical methods have been reported on stability-indicating assay by HPLC method[18,19]. However, there is no stability-indicating fast LC (RRLC) method for simultaneous estimation of IRB and its related impurities in IRB bulk drug samples. The present research work is focused to develop the simple and rapid analytical procedure, which could serve as stability indicating assay method for simultaneous estimation of IRB and its pharmacopoeia specified impurities, along with process related, intermediate and degradation impurities. The present method can reduce the analysis time, manpower and instrument occupancy, effluent load and also significantly reduce analysis cost in routine analysis.

The developed method was validated with respect to International Conference on Harmonisation (ICH) requirements. The present validated stability indicating method can be used as an alternative for routine quality control analysis and stability study of API test samples.

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MATERIALS AND METHODS

IRB and six impurity standards and API test samples were obtained from ecoLogic Technologies Limited, Hyderabad, India. Chemical structure of IRB and known impurities are shown in fig. 1 and these are confirmed by ^{1}HNMR, mass spectroscopy data. Acetonitrile and ortho-phosphoric acid (HPLC grade), sodium hydroxide (NaOH), hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2) GR grade chemicals were purchased from Merck Fine Chemicals, Mumbai, India. Milli Q water is obtained from Millipore direct 8 l/h system.

Agilent infinity series RRLC system consisting quaternary solvent delivery pump, a degasser, an auto injector, column thermostat and photo diode array detector with open lab CDS chemstation and EZ-Chrom software (Agilent Technologies, Clara, US) was used for method development and subsequent validation study.

Optimization of chromatographic conditions:

The main target of the chromatographic method is to get the separation of above said known impurities and degradation products generated during stress studies from the analyte peak. Initially, USP method was attempted to separate the process related impurities along with USP specified impurities. During the analysis, impurity-A and B co-elution was observed by performing USP method condition. It is necessary to know the amount of other process related impurities in bulk drug sample during manufacturing of IRB commercial process. Indeed, the method should be optimized to monitor the impurities during manufacturing of IRB process samples. The present method is developed for the above uncertainty results of impurity-A and B and similarly late eluted other known, unknown impurities in given samples. Various buffer pH, gradient conditions and columns were chosen for method development and optimization. Finally, succeeded on Kromasil C8 3.5 µm, 150×4.6 mm (Kromasil, Brewster, NY) HPLC short column with mobile phase consisting A: ortho phosphoric acid 0.05% v/v and B: acetonitrile, using gradient elution program T (min)/% B: 0/30, 6/55, 9/65, 12/80, 15/80, 16/30, 20/30. Column flow rate was operated at the rate of 1.0 ml/min, injection volume was 5.0 µl and detector was set at 220 nm. The column thermostat temperature was maintained at 35°. Acetonitrile was used as diluent for standard and sample preparations.

Preparation of standard and test sample solutions:

Standard and test solution of IRB were prepared at

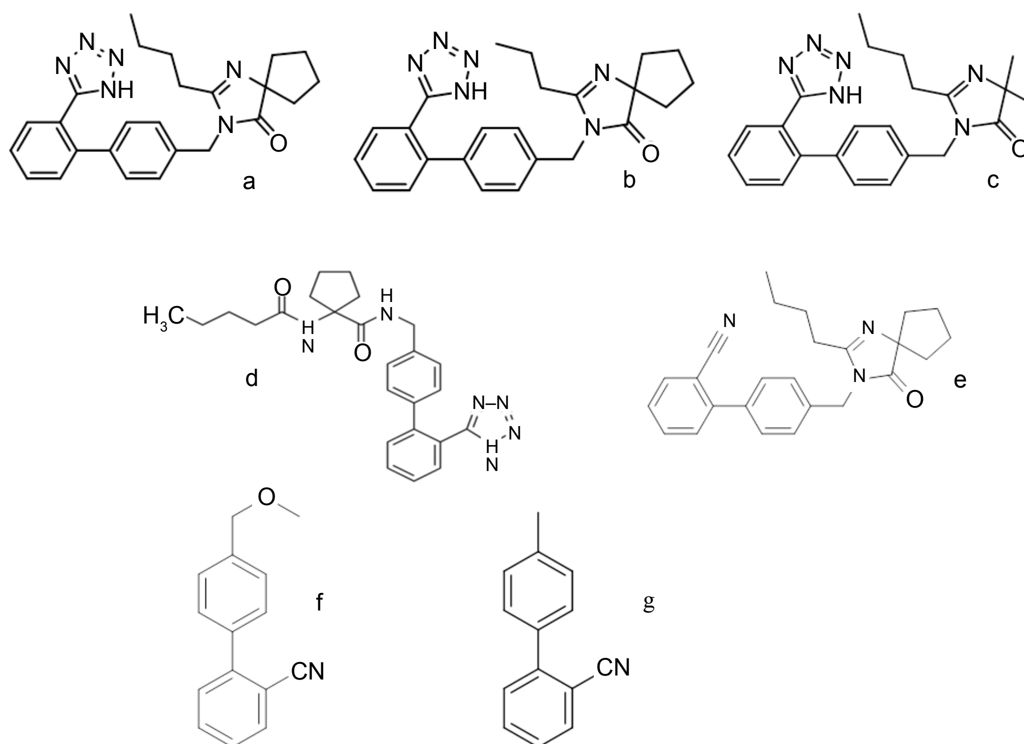

Fig. 1: Irbesartan and its impurities.
(a) Irbesartan; (b) 3-((2'-(1H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-2-propyl-1,3-diazaspiro[4.4]non-1-en-4-one(process impurity); (c) 3-((2'-(1H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-2-butyl-5,5-dimethyl-3,5-dihydro-4H-imidazol-4-one (process impurity); (d) N-((2'-(1H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-1-pentanamidocyclopentane-1-carboxamide(process impurity and USP listed carboxamide impurity); (e) 4'-((2-butyl-4-oxo-1,3-diazaspiro[4.4]non-1-en-3-yl)methyl)-[1,1'-biphenyl]-2-carbonitrile (Key intermediate compound); (f) 4'-(methoxymethyl)-[1,1'-biphenyl]-2-carbonitrile (Process impurity); (g) 4'-methyl-[1,1'-biphenyl]-2-carbonitrile (Carry forward impurity)

concentration of 500 µg/ml using diluent for assay determination and the same solution was used for purity determination. Standard stock solutions of impurities were prepared at concentration of 100 µg/ml and further diluted to 0.5 µg/ml level and spiked in test sample for system suitability evaluation. The same impurity stock solutions were used for related substances method validation study to determine the known impurities with respect to IRB API test sample solutions. A composite sample of IRB API test sample was taken for the entire study.

RESULTS AND DISCUSSION

The specificity of the developed LC method for IRB was carried out in the presence of its six impurities. Stress studies were performed at an initial concentration of 500 µg/ml of IRB API test sample, to provide an indication of stability indicating property and specificity of the proposed method. Acidic and basic stress were performed in 1N HCl and 1N NaOH at 60° for 12 h, respectively. Oxidation study was carried out at 60º in 3% hydrogen peroxide for 12 h. Photo degradation studies were carried out according to Option 2 of Q1B in International Conference on Harmonisation of Technical Requirements for registration of pharmaceuticals for human use guidelines. The drug sample was exposed to light and overall illumination of 1.2 million lux h and an integrated near ultraviolet energy of 200 W.h/m^2. The drug sample was exposed to dry heat at 80° for 10 days to evaluate the ability of the proposed method to separate IRB from its degradation products. Photodiode array detector was employed to ensure the homogeneity and purity of IRB peak in the entire stressed sample solutions. Assessment of mass balance in the degraded samples was carried out to confirm the amount of impurities detected in stressed samples and matched with the amount present before the stress. The mass balance (% assay+% sum of all impurities+% sum of all degradation products) was tabulated in Table 1. According to stress study data (Table 1), product degradation is very less for the duration of stress study performed. The peak purity for IRB peak was passing in all the stressed samples and also there was no interference from degradation products from the analyte peak. Quantitative determination of IRB was carried out for all the stressed samples against qualified working standard.

The selectivity of the method was established from the resolution of IRB from the nearest peak and also among all the other peaks. System suitability results are depicted in Table 2. Typical blank and selectivity chromatograms are shown in figs. 2A and 2B. All the impurities were separated well and from analyte as well with a resolution greater than 1.8. Hence, the method was proved selective. There is no interference was observed from the blank peaks. Peak homogeneity test result is satisfying the requirement during peak purity measurement (Table 2).

Typical method sensitivity LOD and LOQ chromatograms are shown in figs 2C and 2D.. The LOD and LOQ for IRB and its impurities were determined at a signal to noise ratio of 3:1 and 10.1, respectively. By injecting a series of dilute solutions with known concentrations. The obtained LOQ

TABLE 1: RESULTS OF STRESS STUDIES

Stress condition	Time	Assay (%w/w)	Total impurities (%area)	Mass balance (%w/w)
Acid hydrolysis using 1N HCl (at 60°)	12h	99.8	0.35	100.2
Base hydrolysis using 1N NaOH (at 60°)	12 h	99.8	0.34	100.1
Oxidative degradation using 3% H_2O_2	12 h	99.7	0.48	100.2
Photolytic degradation-controlled	11 days	99.5	0.34	99.8
Photolytic degradation-uncontrolled	11 days	99.1	0.34	99.4
Thermal degradation at 80°	10 days	99.3	0.34	99.6

TABLE 2: SYSTEM SUITABILITY RESULTS

Name	Retention time (t_R) in min.	Resolution (R_s) by tangent	USP theoretical plates	Tailing factor (T)
Impurity-A	5.20	-	36116	0.96
Impurity-B	5.37	1.8	38955	1.10
IRB	5.88	3.7	19295	1.37
Impurity-C	7.80	13.5	75837	0.98
Impurity-D	8.38	5.3	101288	1.01
Impurity-E	10.42	16.3	84300	0.98
Impurity-F	12.08	11.6	111955	1.00

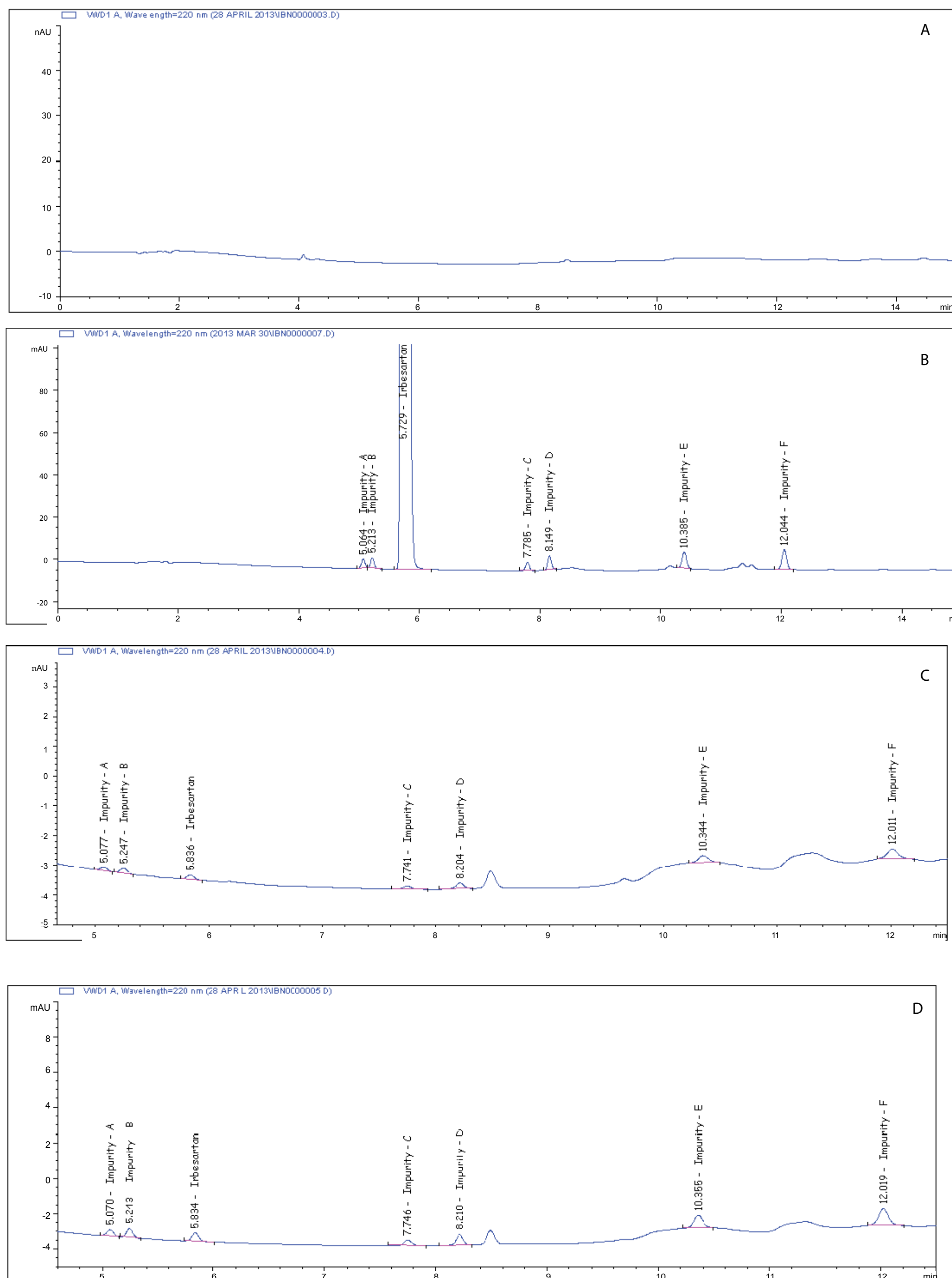

Fig. 2: Typical chromatograms
A. blank chromatogram, B. system suitability chromatogram, C. chromatogram depicting limit of detection and D. chromatogram representing limit of quantification.

concentration is < 0.06 µg/ml. Precision study was also carried out at the LOQ level by injecting six (n=6) individual preparations and calculating the RSD percentage of the area (Table 3). The observed %RSD value is below 2.6 in method precision study and below 2.1 in intermediate precision study (Table 3).

Assay method precision, intermediate precision and similarly related substances method precisions were shown in Table 3. Linearity test solutions for the assay method were performed from 125 to 1000 µg/ml (i.e., 125, 250, 375, 500, 750 and 1000 µg/ml). The responses were measured as peak areas and plotted against concentration. Assay method precision was carried out using six independent test solutions and a standard preparation. The intermediate precision of assay method was also evaluated using different instruments on different

days. Similarly, related substances method precision and intermediate precision was also carried out using six independent test solutions containing 0.2% level of known impurities with respect to the test sample concentration (i.e., 500 µg/ml) (Table 3).

Similarly, linearity test solutions for the related substances (RS) method were performed from LOQ to 2 µg/ml of impurity level (i.e., LOQ to 0.4% impurity level with respect to the test conc. 500 µg/ml). The calibration curve was drawn by plotting the each impurity peak area versus its corresponding concentration. Both the methods (RS and Assay) are showing good correlation coefficient >0.999 and it indicates existence of an excellent correlation between the peak area and concentration of IRB and six impurities. The obtained linearity experiment results are given in Table 3.

The accuracy of the assay method was evaluated in triplicate using three concentration levels such as 250, 500 and 750 µg /ml (i.e., 50, 100 and 150% level of assay test concentration) and the percentage of recoveries of IRB were calculated at each level and the % recovery is 99.6 to 100.7%. Related substance method accuracy was also carried out in triplicate using three concentration levels of 0.5, 1 and 1.5 µg/ml (i.e., 0.1, 0.2 and 0.3% levels of impurities with

TABLE 3: LOD, LOQ, REGRESSION AND PRECISION DATA

Parameter	IRB	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Imp-F
LOD (µg/ml)	0.019	0.018	0.018	0.017	0.017	0.016	0.018
LOQ (µg/ml)	0.064	0.059	0.058	0.056	0.056	0.054	0.06
Regression equation							
Slope (m)	33973.5	30589.7	42613.7	30708	50500.6	75940.7	88045.2
Intercept (c)	-486.7	-1738.2	-2090.5	-1595	-2276.6	-3271.6	- 3337.4
Correlation coefficient	0.9997	0.9998	0.9998	0.9992	0.9996	0.9993	0.9995
Y-intercept at 100% level	-1.2%	-5.4%	-4.6%	-5.5%	-4.5%	-4.8%	-3.4%
Method precision[a]	0.28%	0.28%	0.33%	0.31%	2.2%	2.6%	0.35%
Intermediate precision[a]	0.54%	0.82%	0.35%	0.98%	1.9%	2.1%	0.45%

[a]Six determinations of specified level impurities with respect to analyte concentration (500 µg/ml) 100 µg/ml for assay of IRB.

TABLE 4: RESULTS OF ACCURACY FOR RELATED SUBSTANCE AND ASSAY

Name	Level (%)	Amount added in µg/ml	Amount recovered in µg/ml	%Recovery
Impurity-A	50	0.564	0.544	96.5
	100	1.128	1.080	95.7
	150	1.691	1.590	94.0
Impurity-B	50	0.564	0.535	94.9
	100	1.128	1.035	91.8
	150	1.692	1.591	94.0
IRB[a]	50	0.627	0.569	90.7
	100	1.255	1.178	93.9
	150	1.882	1.665	88.5
Impurity-C	50	0.503	0.474	94.2
	100	1.007	0.972	96.5
	150	1.510	1.385	91.7
Impurity-D	50	0.534	0.515	96.4
	100	1.068	1.006	94.2
	150	1.601	1.498	93.6
Impurity-E	50	0.553	0.535	96.7
	100	1.105	1.093	98.9
	150	1.658	1.597	96.3
Impurity-F	50	0.589	0.577	98.0
	100	1.179	1.141	96.8
	150	1.658	1.622	97.8
IRB[b]	50	258.96	257.96	99.6
	100	507.81	508.11	100.1
	150	753.86	759.24	100.7

[a]Unknown impurity level of IRB with respect to analyte concentration (500 µg/ml),
[b]Assay of IRB concentration (500 µg/ml).

TABLE 5: RESULTS OF ROBUSTNESS EVALUATION

Chromatographic changes	Resolution[a]	Tailing factor	Theoretical plate count
Flow rate (ml/min)			
0.8	1.8	1.21	22733
1.2	1.5	1.41	16146
Temperature (°)			
30	1.6	1.40	17611
40	1.7	1.37	19846
Wavelength (nm)			
218	1.6	1.43	17988
222	1.6	1.36	19690
Mobile phase composition (%)			
67:33	1.7	1.51	13389
27:73	1.6	1.18	23165

[a]Resolution measured between Impurities-A and B peaks

respect to the test concentration of 500 µg/ml) and the % recovery is 88.5 to 98.9% (Table 4).

Accuracy parameter is performed to determine the closeness of test results with that of the true value which is expressed as %recovery. The results of accuracy were depicted in Table 4. Robustness of the method was determined as a measure of the analytical method capability to be unaffected by small variations in method parameters (Table 5). The robustness was determined by the variation of flow rate by ± 0.2 ml/min, column temperature by ±5°, composition of mobile phase by ±10% (in terms of organic component) and slight variation in wavelength by ±2 nm. At these changed conditions, the system suitability was evaluated at each condition. In all the conditions, the resolution between critical pair was greater than 1.7 and tailing factor of IRB peak was found be less than or equal to 1.5 (Table 5).

The %RSD of assay of IRB during solution stability and mobile phase stability experiments is less than 1.0%. No significant changes were observed in the content of impurity-1, impurity-2, impurity-3, impurity-4, impurity-5 and impurity-6 during solution stability and mobile phase stability experiments. The solution stability and mobile phase stability experiments data confirms that the sample solutions, mobile phase used for assay and related substances determination are stable up to the study period of 48 h.

The current stability-indicating RRLC method was found to be suitable for the determination of assay of IRB and its related impurities. The developed method is simple, specific and rapid, and the method was fully validated as per regulatory requirements i.e., ICH and USP. The present method can be successfully used for the quality determination of IRB in commercial manufacturing batches and also it for stability monitoring (accelerated, long term stability studies) in quality control laboratories. Most importantly, the established method is greener than other published methods in terms of analysis cost, time and effluent load at laboratories.

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CONFLICT OF INTERESTS

None declared.

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