Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Ramipril, Aspirin and Simvastatin in Bulk and Pharmaceutical Dosage Form

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ABSTRACT :

Objective: The objective of present work was to develop and validate a simple, fast, precise, selective and accurate RP-HPLC method for the simultaneous determination Ramipril(RAM), Aspirin (ASP) and Simvastatin (SIM) in a Pharmaceutical dosage form.

Materials and Methods: The separation of these three drugs was achieved on a SHISHEDO C18, 250×4.6mm, 5 micron size column with a mobile phase consisting of 0.5% Ortho phosphoric acid :ACN:Methanol(20:10:70 v/v) at a flow rate of 1ml/min and UV detection at 226nm.

Results: The retention times were observed to be 2.1, 2.7 and 9.6 minutes for Ramipril (RAM), Aspirin (ASP) and Simvastatin (SIM) respectively. Linearity was found to be 5-15µg/ml, 50-150µg/ml, 20-60µg/ml for Ramipril(RAM), Aspirin(ASP)and Simvastatin(SIM) respectively. The method was statistically validated for linearity, recovery, limit of detection, limit of quantification, accuracy and precision. The stress testing of the drugs individually and their mixture is carried out under acidic, alkaline, oxidation, photo-stability and thermal degradation conditions and its degradation products are well resolved from the analyte peaks

Conclusion: This method was successfully validated for accuracy, precision, and linearity, limit of detection and limit of quantification.

Keywords: Ramipril(RAM), Aspirin (ASP) Simvastatin (SIM)reverse phase high performance liquid chromatography.

INTRODUCTION:

Ramipril (RAM), is chemically (2s, 3as, 6as)-1-[(2s)-2-{[(2s)-1-ethoxy-1-oxo -4- phenyl butan-2-yl] amino} Propanoyl]-Octahydro Cyclopenta [b] pyrrole-2-carboxylic acid (Fig.1) It is an Angiotensin-converting enzyme inhibitor, Anti-hypertensive agents.

Aspirin (ASP) is chemically 2-(acetyloxy) benzoic Acid (Fig.2). It is an Anti-inflammatory agents, Antipyretics, Aggregation inhibitors , Salicylates

Simvastatin (SIM) is chemically (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}- 3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2- dimethylbutanoate (Fig.3). It is an Anticholesteremic Agents, Hydroxymethylglutaryl-CoA Reductase Inhibitors, Hypolipidemic Agents.

Detailed literature survey revealed analytical methods like Spectrophotometric [4, 5, 6], HPTLC [7-10] are available for the estimation of these drugs individually or in combination with other drugs. But very few RP-HPLC methods [11-26] are available for the simultaneous estimation of these drugs. Hence, we tried to develop simple RP-HPLC methods for the simultaneous estimation of these drugs. The developed methods were validated as per the guidelines of ICH [24]. To establish Stability Indicating [25] natures of the RP-HPLC method, forced degradation of drug substances was performed under stress conditions (peroxide, acid, base, thermal, ultraviolet and neutral hydrolysis).

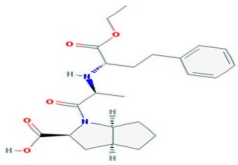

Fig.1 Chemical Structure of Ramipril

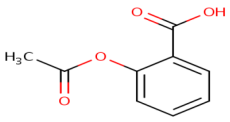

Fig.2 Chemical Structure of Aspirin

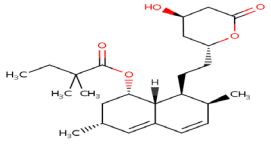

Fig.3 Chemical Structure of Simvastatin

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Conflict of interest: Authors reported none

MATERIALS AND METHODS

Chemicals and reagents

Ramipril(RAM), Aspirin(ASP) and Simvastatin(SIM), working standards were procured from Dr. Reddys Laboratories Ltd. Commercially available as TRINOMIA tablets were purchased from the local pharmacy. HPLC grade acetonitrile and methanol were purchased from Merck Specialities Pvt. Ltd., Mumbai. HPLC grade water was purchased from Thermo Fisher Scientifics Ltd., Mumbai. Orthophosphoric acid, hydrochloric acid, sodium hydroxide pellets purified and hydrogen peroxide 30% of AR grade were procured from Merck Specialties Pvt. Ltd., Mumbai.

Instrumentation and analytical conditions

RP-HPLC method was performed on the HPLC system (Shimadzu) consisting of binary gradient pump and UV detector (LC-20AD) was employed for analysis and rheodyne injector with 20µl fixed loop was used for the present study.

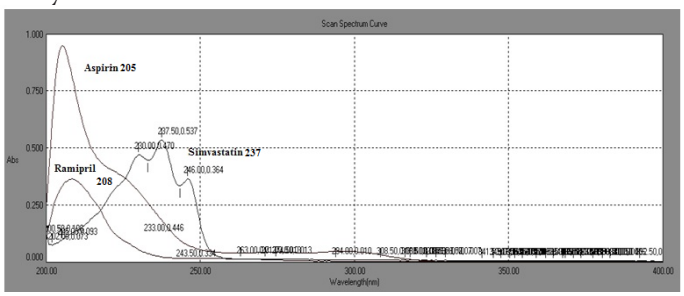

Fig.4 Overlay spectrum of RAM, ASP and SIM.

Preparation of solutions:

Preparation of standard solutions

Standard stock solution of Aspirin, Ramipril and Simvastatin were prepared by transferring accurately weighed ASP (10mg), RAM (10mg) and SIM (10mg) in to a 10ml volumetric flask separately,to this add 0.1ml HCL (0.1N) and diluted to a mark with methanol, to obtain a standard solution of ASP (1000 µg/ml) RAM (1000µg/ml) and SIM (1000µg/ml). From these solutions, standard stock solutions were prepared in a 10 ml volumetric flasks and made upto the volume with mobile phase (Orthophosphoric acid: Acetonitrile : Methanol 20:10:70 v/v) to get the concentration of 100µg/ml of ASP, 100µg/ml of RAM and 100µg/ml of SIM.

Preparation of the sample solutions

20 tablets were taken and their average weight was calculated, tablets were crushed to fine powder and dose equvivalent to 10 mg of ASP, RAM and SIM were taken into 10 ml volumetric flask and add 0.1ml HCL(0.1N) diluted up to the mark with methanol, to obtain a concentration of 1000 µg/ml of ASP, RAM, SIM. 1 ml of the above solution were taken in a 10ml volumetric flasks and diluted to 10 ml with mobile phase (0.5%orthophosphoric acid:acetonitrile:Methanol 20:10:70 v/v) to obtain a concentration of 100 µg/ml of ASP, RAM and SIM.. From 100µg/ml solution of ASP, RAM, SIM pipette out 1ml of ASP, 0.1ml of RAM and 4ml of SIM and make up to the final volume with mobile phase.

Optimized analytical methods

Shiseido C18 column (250mm X 4.6mm I.D.) was used as stationary phase. RAM, ASP and SIM were eluted isocratically with a flow rate of 1.0 ml/min using a mobile phase consisting of 0.5% Ortho phosphoric acid :ACN:Methanol (20:10:70 v/v) respectively. The wavelength of the UV detector was set at 226nm. The prepared mobile phase was filtered through 0.45 µm membrane filter (Millipore) and sonicated before use.

METHOD VALIDATION

The developed methods were validated according to International Conference on Harmonization guidelines for validation of analytical procedures.Validation was done as per ICH guideline Q2 (R1). The developed method was validated with respect to parameters such as linearity, LOD and LOQ, precision, accuracy and specificity.

System suitability

The system suitability of the HPLC method was determined by making six replicate injections from freshly prepared standard solutions and analyzing each solute for their retention time, theoretical plates number (N) and tailing factors (T).

Specificity

It is the ability to assess unequivocally the analyte in the presence of impurities, degradants and matrix. To determine this, 20µl of blank, standard and sample solutions were injected separately in triplicate and respective chromatograms were recorded under the optimized conditions.

Linearity

The calibration curves were obtained with concentrations of the standard solutions of 5-15 µg/ml, 50-150µg/ml and 20-60 µg/ml for RAM, ASP and SIM respectively. Linearity was evaluated by regression analysis, which was calculated by the least square regression method.

Accuracy

To check the degree of accuracy of recovery studies were performed in triplicate by the standard addition method at 50%, 100% and 150% levels.

Precision

Precision was checked by analyzing the samples at different time intervals of the same day (intra-day precision) as well as on different days (inter-day precision).

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the values of slopes and intercepts of the calibration curves for three drugs.

Robustness

Robustness was determined by analysis of samples under deliberately changed chromatographic conditions. The flow rate of the mobile phase was changed from 0.9 ml/min to 1.0 ml/min and 1.1 ml/min. The ratio of the organic phase was changed by 2%, i.e., 78%, 80%, 82%. The effect of retention time and peak parameter was studied.

Assay of Pharmaceutical dosage form

20 µL of each standard and sample solution were injected and from the peak areas of RAM, ASP and SIM amount of each drug in samples was computed.

Stability studies

Degradation studies were performed in sample solutions containing 10 µg/ml of RAM, 100µg/ml of ASP and 40 µg/

ml of SIM.

Acidic degradation

1 ml of 0.1 M HCl were added individually to the final drug solution in different volumetric flasks and they were refluxed for 1 hr at 60°C. After 1 hr., these solutions were injected under optimized chromatographic conditions.

Alkaline degradation

1 ml of 0.1 M NaOH were added individually to the final drug solution in different volumetric flasks and they were refluxed for 1 hr at 60°C. After 1 hr., these solutions were injected under optimized chromatographic conditions.

Oxidative degradation

1 ml of 3% H2O2 were added individually to the final drug solution in different volumetric flasks and they were refluxed for 1 hr at 60°C. After 1 hr., these solutions were injected under optimized chromatographic conditions.

Photolytic degradation

The final drug solution was kept at room temperature and exposed to sunlight for 8 hrs. After 8 hrs, this solution was injected under optimized chromatographic conditions.

Thermal degradation

The final drug solution was kept at a temperature of 60°C for 6 hrs. After 6 hrsThis solution was injected under optimized chromatographic conditions.

RESULTS AND DISCUSSION

Method development

The HPLC procedure was optimized for simultaneous determination of RAM, ASP and SIM. Good resolution of both the components was obtained with 0.5% Ortho phosphoric acid :ACN:Methanol (20:10:70 v/v) . The flow rate of 1 mL/min was optimum. UV detection was made at 226nm. At this wavelength ASP, RAM and SIM can be quantified. Hence, 226 nm determined empirically has been found to be optimum. The average retention times for RAM, ASP and SIM was found to be 2.1, 2.7 and 9.6 min, respectively.

System suitability

Twenty micro liters of working standard solution containing 10µg/ml of RAM, 100µg/ml of ASP and 40µg/ml of SIM was prepared and injected into the system under optimized chromatographic conditions. Chromatograms were recorded and studied for different system suitability parameters like tailing factor, theoretical plates, resolution and peak area, peak heights were also studies. Six different working standard solutions were injected to study this parameter and all the suitability parameters were found to be within the limits. The system suitability parameters were shown in table 1.

Table.No. 1 System Suitability Parameters of RAM, ASP, SIM.

Parameter	RAM	ASP	SIM
Retention Time	2.1	2.7	9.6
No. of Theoretical plates	2840.3	4790.1	8826.6
Tailing Factor	1.132	1.125	1.143

**Average of six readings*

Specificity

The HPLC chromatograms were recorded for blank and sample under optimized analytical conditions, compared them with that of standard solution, and found no additional peaks. The two peaks were completely separated in HPLC chromatogram and the resolution was found to be more than 2. Even in presence of excipients of the sample no interfereing peaks were found in HPLC chromatogram.

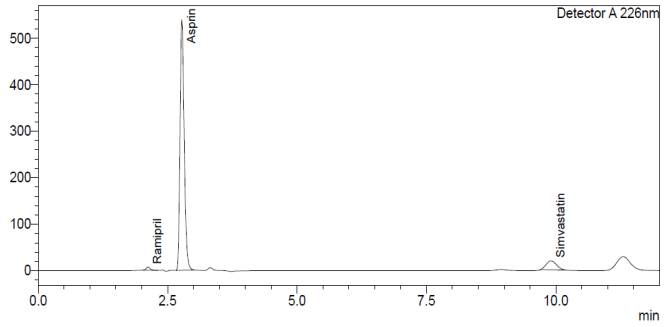

Fig. 5 Chromatogram of well resolved peaks of RAM, ASP and SIM

Linearity

For HPLC method, the calibration curves of RAM, ASP and SIM were constructed in the concentration range of 5-15 µg/ml, 50-150 µg/ml and 20-60µg/ml of RAM, ASP and SIM respectively. The plots obtained from linear regression and residuals analysis are given below.

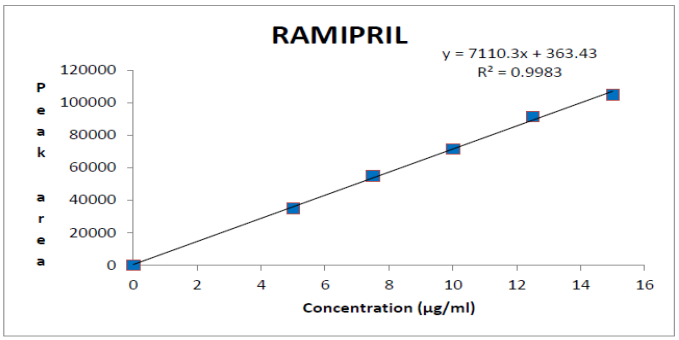

Fig. 6 Graph of Linearity of RAM

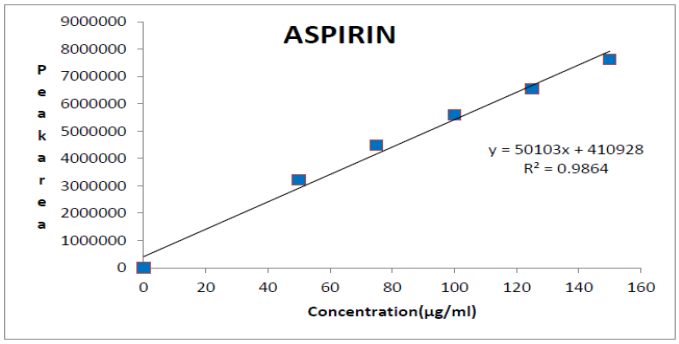

Fig. 7 Graph of Linearity of ASP

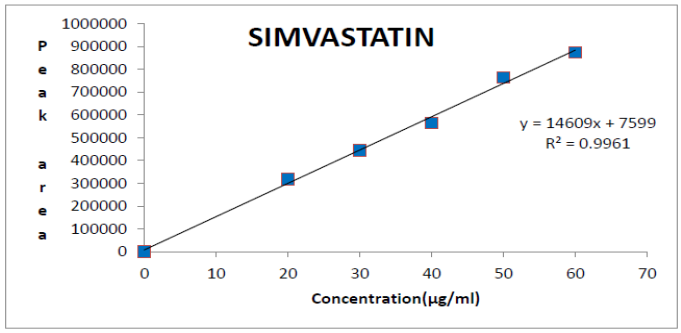

Fig. 8 Graph of Linearity of SIM

Table. No. 2 Linearity of RAM, ASP and SIM

Parameter	RAM	ASP	SIM
Regression equation	Y=7110.3x+363.43	Y=50103x+410928	Y=14609x+7599
Linearity (µg/ml)	5-15µg/ml	50-100µg/ml	20-60µg/ml
Correlation coefficient(R^2)	0.9983	0.9864	0.9961

Accuracy

The accuracy for proposed method was determined, recovery studies were performed in mentioned levels and recorded (Table 3),Obtained results were found to be within the limits of 98-102%, indicating an agreement between the true value and found value.

Table. No. 3 Accuracy of RAM, ASP and SIM

Drug	Recovery			%RSD		
	50%	100%	150%	50%	100%	150%
RAM	99.74	99.69	99.67	1.015	1.849	1.484
ASP	99.76	99.97	99.90	1.616	1.004	0.722
SIM	99.88	99.89	99.84	0.661	1.452	0.693

Precision

Precision was calculated as intra-day and inter-day variations for the drugs. Percent relative standard deviations for estimation of RAM, ASP and SIM under intra-day and inter-day variations were found to be less than 2 (Table 4).

Table. No. 4 Precision of RAM, ASP and SIM

Drug	Concentration (µg/ml)	Intraday (%RSD)	Interday (%RSD)
RAM	50%	0.240	1.268
	100%	0.657	1.016
	150%	1.324	0.277
ASP	50%	0.846	0.574
	100%	0.616	0.571
	150%	0.126	1.195
SIM	50%	0.362	1.136
	100%	0.766	0.620
	150%	0.3133	1.300

Limit of detection (LOD) and Limit of quantitation (LOQ)

The LOD and LOQ were calculated according to the 3.3 σ/s and 10 σ/s criteria, respectively; where σ is the standard deviation of the peak area and s is the slope of the corresponding calibration curve.

Table. No. 5 LOD and LOQ of RAM, ASP and SIM

Drug	LOD (µg/ml)	LOQ (µg/ml)
RAM	1	5
ASP	0.1	10
SIM	0.8	2.5

Robustness

For robustness studies, conditions like flow rate and concentration of organic phase were changed and method was performed. In all deliberately varied conditions, percent relative standard deviations for peak areas, retention times, theoretical plates and tailing factor were found to be less than 2% (Table 6).

Table. No. 6 Robustness Parameters of RAM, ASP and SIM

S.No.	Parameter	RAM Rt(min)	ASP Rt(min)	SIM Rt (min)
1	Initial Flow	2.1	2.7	9.5
2	Flow 0.9 ml/min	2.3	3.1	11.4
3	Flow 1.1 ml/min	1.8	2.5	9.0
4	Initial Oraganic phase conc	2.0	2.7	9.7
5	Organic phase, 2% less	2.1	2.7	8.5
6	Organic phase, 2% more	2.0	2.7	11.3

Assay

The percent of assay was calculated using absorbances by using peak areas of standard and sample. The experimental values obtained for the determination of RAM, ASP and SIM in Pharmaceutical formulation were within the claimed limits (Table 7).

Table. No. 7 Assay data of marketed formulation

Drug	Amount labeled (mg)	Amount found	% Assay
RAM	10	99.5mg	99.5%
ASP	100	9.85mg	98.5%
SIM	40	39.5mg	98.75%

Stability Studies

The following degradation results were found when RAM, ASP and SIM were subjected to,

Acidic degradation:SIM showed good stability in acidic conditions compared to ASP and RAM. RAM showed more degradation in 3rd day compared to 1st and 2nd day. Chromatogram of Acidic condition can be seen in fig. 9 for RAM, fig. 10for ASP and in fig. 11 for SIM.

Alkaline degradation: ASP showed more degradation in basic conditions than RAM and SIM. ASP showed more degradation on 3rd day. Chromatogram of Basic condition can be seen in fig. 9 for RAM, fig. 10for ASP and in fig. 11 for SIM.

Oxidative degradation: All the three drugs showed good stability in Oxidative condition. Chromatogram of Oxidative condition can be seen in fig. 9 for RAM, fig. 10for ASP and in fig. 11 for SIM.

Photolytic degradation: All the three drugs showed good stability under photolytic conditions with very less degradation. Chromatogram of Photolytic condition can be seen in fig. 9 for RAM, fig. 10 for ASP and in fig. 11 for SIM.

Thermal degradation: All the three drugs showed good stability under thermal conditions with very less degradation. Chromatogram of the thermal condition can be seen in fig. 9 for RAM, fig. 10for ASP and in fig. 11 for SIM.

The percent amount of drug degraded after degradation studies and the Rt of degradation products are given in (Table 8, 9, 10). The pattern of degradation of the drugs individually in all the conditions and in different days was well portrayed in the Fig. 12,13,14. In the proposed HPLC method, different proportions of acetonitrile and orhophosphoric acid (OPA) were tried for selection of the mobile phase. Ultimately, 0.5% OPA in water and acetonitrile in a proportion of 70:30 v/v respectively was finalized as the mobile phase. The elution order was RAM (Rt=2.1 min), ASP (R_t=2.7min) and SIM (Rt=9.6 min), at a flow rate of 1.0 ml/min. The chromatogram was recorded at 226nm. The developed method was validated as per ICH guidelines. Parameters like precision, accuracy, specificity, ruggedness, robustness were done and found to be within the acceptance criteria. LOD and LOQ were determined and the developed method was applied for determination of assay of TRINOMIA Tablets. The stability of the drugs was examined under different stress conditions such as acidic, basic, peroxide, thermal and under UV conditions.

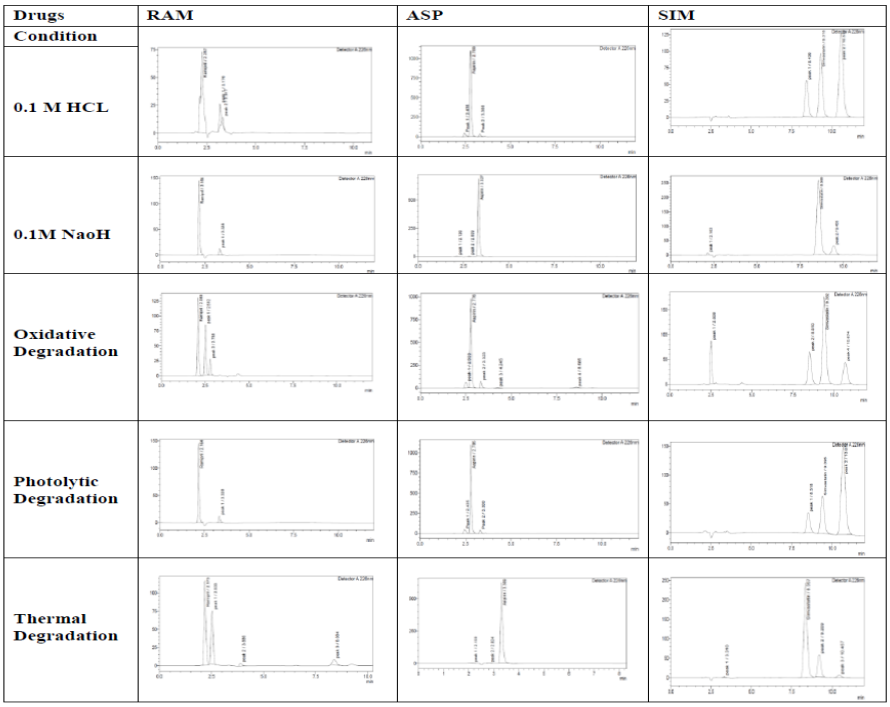

Fig. 9 Chromatograms of RAM, ASP, SIM in Different stress conditions

Day	Condition	% Degraded ± SD of RAM	% Degraded ± SD of ASP	% Degraded ± SD of SIM
1st Day	Acid	12.14±7.60	10.26±2.10	7.19±2.01
	Base	15.20±2.08	35.28±2.66	16.69±1.83
	Oxidative	3.43±1.52	10.89±2.51	19.18±2.58
	Photolytic	5.26±2.09	14.82±1.40	21.26±2.01
	Thermal	10.32±1.92	19.93±2.06	4.81±1.83
3rd Day	Acid	40.75±2.10	16.41±2.06	15.46±1.87
	Base	25.24±2.31	40.12±1.49	24.23±2.07
	Oxidative	15.57±2.05	16.80±2.51	32.15±2.04
	Photolytic	12.84±2.05	21.26±2.01	32.29±1.69
	Thermal	27.74±1.81	24.36±2.02	31.11±2.02
5th Day	Acid	67.51±2.28	24.72±2.13	20.65±2.04
	Base	35.83±1.99	43.15±1.99	25.06±1.99
	Oxidative	27.42±2.53	26.54±1.94	36.18±2.59
	Photolytic	19.79±2.70	32.74±1.90	40.63±0.99
	Thermal	41.61±2.14	27.44±2.52	31.28±2.11

Table.No. 8 Degradation data of RAM, ASP, SIM in different conditions

CONCLUSION

The proposed RP-HPLC were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are low, indicating a high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. The RP-HPLC method could selectively quantifies RAM, ASP and SIM in presence of its degradation products hence; it can be employed as a stability indicating method. From the found experimental data it can be concluded that the developed stability indicating chromatographic methods are accurate, precise and selective and can be employed successfully for the estimation of RAM, ASP and SIM in Pharmaceutical dosage form.

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