Original Article

Development Of An LC-MS/MS Approach To Detect And Quantify Two Impurities Of Ranolazine

Ravi Uppala[1], Manivannan M[2], Arthanareeswari. M[3]*,Venkatasubbaiah. B [4]

[1,3] SRM Institute of Science and Technology, Kattankulathur, Department of Chemistry, Tamilnadu, 603203, India.
[2]Department of Chemistry, Centre for Research & Development, PRIST Deemed University, Thanjavur -613403, Tamil Nadu, India.
[4] Dr Reddys laboratories limited, Analytical Research, Hyderabad 500 072, India.

***Corresponding Author:** Arthanareeswari. M
Department of Chemistry, Centre for Research & Development, PRIST Deemed University, Thanjavur -613403, Tamil Nadu, India.
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Abstract

In the ranolazine (RLE) synthesis 1-Methoxy-2-(oxiranyl methoxy) benzene (RC-A) and 2-Chloro-N-(2,6-dimethylphenyl) acetamide (RC-B) are generated as an intermediates. The presence of RC-A and RC-B impurities in RLE could potentially affect its effectiveness. The purpose of this investigation was to establish a LC-MS/MS methodology to identify and evaluate RC-A and RC-B impurities in RLE samples. The method for RC-A and RC-B analysis was developed on X-Select CSH C18 column with gradient elution using mobile phase consisted of 0.1% ammonia (mobile phase A) and methanol (mobile phase B). Mass spectrometer with electrospray ionization operated in the MRM mode was used in the analysis of RC-A (m/z181.1→151.1) and RC-B (m/z198.2→107.1). The LC-MS/MS methodology proposed showed a good linearity (0.251 to 1.128 ppm and 0.258 to 1.141 ppm), good system precision (RSD = 0.8% and 2.9%), good method precision (RSD = 1.0% and 1.3%), acceptable accuracy (94.1-106.0% and 96.2-99.2%), low detection limit (0.075 ppm and 0.077 ppm) and low quantitation limit (0.251 ppm and 0.258 ppm) for RC-A and RC-B, respectively. The LC-MS/MS methodology proposed can be utilized to assess the quality of RLE sample for the presence of RC-A and RC-B impurities.

Index Terms— Process impurities, Ranolazine, LC-MS/MS, X-Select CSH column, MRM mode .

INTRODUCTION

Ranolazine (RLE) is a derivative of piperazine with anti-neoplastic and anti-anginal activity [1,2]. The mechanism of action of RLE for its anti-ischemic activity may involve altering the late transcellular Na^{2+} current in ischemic myocyte. By preventing intracellular Na^{2+} levels from increasing, RLE may affect the transportation function of Na^{2+} dependent Ca^{2+} channels and blocks the Ca^{2+}surplus in myocardial ischemia, thus preventing cellular damage [3,5]. The antineoplastic effect of RLE may rely on its inhibitory action on oxidation of fatty acids which may stimulate tumour cells to apoptosis and decrease the propagation of tumour cells [5].

1-Methoxy-2-(oxiranyl methoxy)benzene (RC-A) and 2-Chloro-N-(2,6-dimethylphenyl)acetamide (RC-B) are intermediates generated during RLE chemical synthesis process [6]. The chemical structures of RLE, RC-A and RC-B are given in Figure 1. Using the Derek nexus software programme, RC-A and RC-B were determined to belong to genotoxic compounds of class 3. The detection and quantitation of RC-A and RC-B throughout production of RLE is extremely difficult. Impurities, RC-A and RC-B, have a significant impact on the purity and effectiveness of RLE. It is also difficult to completely remove RC-A and RC-B from the RLE product. Significant decrease of RC-A and RC-B impurities to the lowest level possible in RLE is therefore important. A novel and valid approach for the detection and quantitation of trace impurities in RLE must therefore be established.

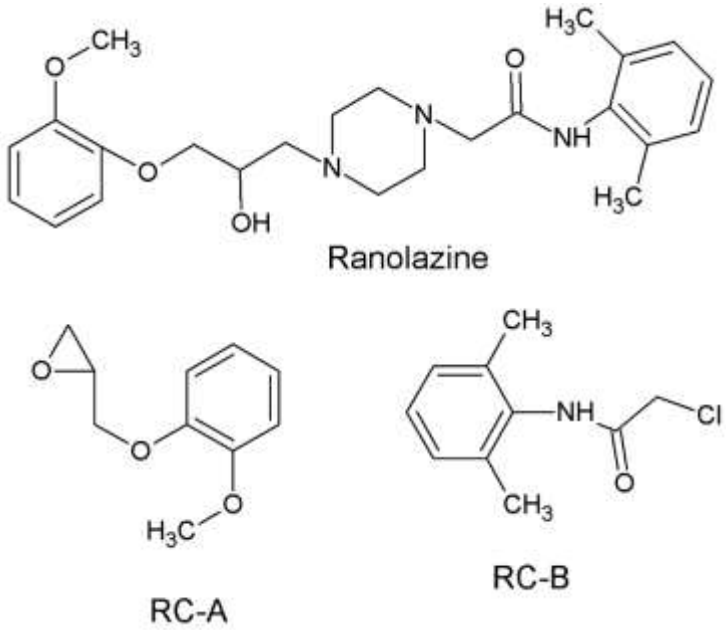


Figure 1 Chemical structures

Malati et al., described a RP-UPLC based method to quantitate fifteen related impurities in RLE using BEH RP 18 column as stationary phase; sodium buffer (7.3 pH), acetonitrile and triethylamine blend as mobile phase in gradient mode of elution system [7]. These fifteen related impurities include preparatory materials of RLE synthesis, positional isomers of TLE, degradants of RLE and by-products generated during RLE synthesis. Madhavi et al., described a stability indicating HPLC based method to quantitate four related impurities in RLE using Inertsil ODS column as stationary phase; sodium buffer (4.5 pH), acetonitrile, water and triethylamine blend as mobile phase in gradient mode of elution system [8]. These four related impurities include intermediates generated during RLE synthesis.

The biggest limitation of HPLC and UPLC for trace-level impurities is the poor sensitivity of the UV detector, that could not satisfy the detection and quantitation needs. To quantify impurities, advanced techniques such as LC-MS, GC-MS, UHPLC-MS/MS are being used because of high sensitivity and selectivity [9-16]. A number of LC-MS [9-12], GC-MS [13-15] and UHPLC-MS/MS [16] methods for the detection and quantitation of impurities in bulk medications and formulations have recently been reported. However, no LC-MS method is reported to detect and quantitate trace levels of RC-A and RC-B simultaneously in the RLE sample. This work was aimed to develop and validate an LC-MS method to detect and quantitate trace levels of RC-A and RC-B simultaneously. After development and validation, the method was applied to detect and quantify RC-A and RC-B in RLE batch samples.

2. Experimental

2.1. Materials

The standard impurities RC-A and RC-B were obtained from *API Pharma Tech Pvt Ltd, Hyderabad, India* and RLE sample of purity 99.85% was also from the same company. The percent purities of RC-A and RC-B were 99.0% and 99.5%, respectively. Milli Q (Bedford, USA) water has been used throughout investigation. Formic acid of AR grade, and acetonitrile, methanol and ammonia of HPLC grade were obtained from Merck (Darmstadt, Germany).

2.2. Instrumentation

AB Sciex LC-MS/MS system model API 4000 (MA, USA), X-Select CSH C18 (100 mm × 3.0 mm, 2.5 µm) column, AB Sciex Analyst software (MA, USA) and Mettler Toledo analytical balance (Switzerland) were employed for analysis of RC-A and RC-B.

2.3. Conditions

The X-Select CSH C18 column and auto-sampler port were operated at temperatures of 45°C and 25°C, respectively for separation. Mobile phases A and B were 0.1% ammonia solution (pH 5.50, adjusted with formic acid) and methanol, respectively. The procedure for gradient elution was as follows: 55% volume of mobile phase A and 45% volume of mobile

phase B from 0 - 7 min; 10% volume of mobile phase A and 90% volume of mobile phase B from 7.1 - 10.0 min; 55% volume of mobile phase A and 45% volume of mobile phase B at from 10.1 - 15.0 min. The flow rate, total analysis time and injection size was 0.3 mL min^{-1}, 15 min and 10 µL, respectively. Water and acetonitrile (20:80, *v/v*) was used for needle wash. For sample preparations, water and acetonitrile (30:70, *v/v*) used as a diluent.

Mass spectrometer fitting with positive type of electro spray ionization operated in the MRM mode was used in RC-A and RC-B analysis. The pressure of collision gas, curtain gas, neubilizing gas and drying gas were set at 5 psi, 20 psi, 50 psi, and 35 psi, respectively. The ion spray voltage and ion source temperature were set at 5000 V and 500°C. The ion transitions for concentration determination were *m/z*181.1→151.1 for RC-A and *m/z*198.2→107.1for RC-B.

2.4. RC-A and RC-B solutions

Stock RC-A and RC-B solution (100 ppm) was prepared in water and acetonitrile (30:70, v/v) solvent blend. Working RC-A and RC-B solution (0.75 ppm) was prepared from stock RC-A and RC-B solution (100 ppm) through apt dilution with water and acetonitrile (30:70, v/v) solvent blend. Calibration RC-A and RC-B solutions with RC-B at 0.251 to 1.128 ppm concentration range and RC-B at 0.258 to 1.141 ppm concentration range were prepared.

2.5. RLE sample solution

A solution of RLE was prepared by direct weighing (400 mg) of RLE substance with subsequent dissolution in water and acetonitrile (30:70, *v/v*) solvent blend by sonication at 26 ºC for 2 min in 10 mL flask. Volume was made after sonication to mark with the same solvent system. The concentration of the RLE sample solution was 4000 ppm.

2.6. Procedure to analyze RC-A and RC-B in RLE samples

Equilibrated the LC-MS system for at least 2 hr. Aliquots (10 µL) of blank diluent (n=1), working RC-A and RC-B solution (n=6) and RLE sample solution (n=2) into the system and analysed with proposed conditions of LC-MS/MS. The RC-A and RC-B contents of the RLE sample were measured using the formula beneath:

$$\text{Impurity content (ppm)} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times P \times 10000$$

Where, AT - average area of RC-A/RC-B in RLE sample solution; AS - average area of RC-A/RC-B in working RC-A and RC-B solution; WS - weight of RC-A/RC-B (mg) in working RC-A and RC-B solution; DS - dilution factor of RC-A and RC-B in working RC-A and RC-B solution; WT - weight of RLE sample (mg); DT - dilution factor of RLE in RLE sample solution; and P - potency of RC-A/RC-B.

3. Results and Discussion

3.1. Method establishment for RC-A and RC-B analysis

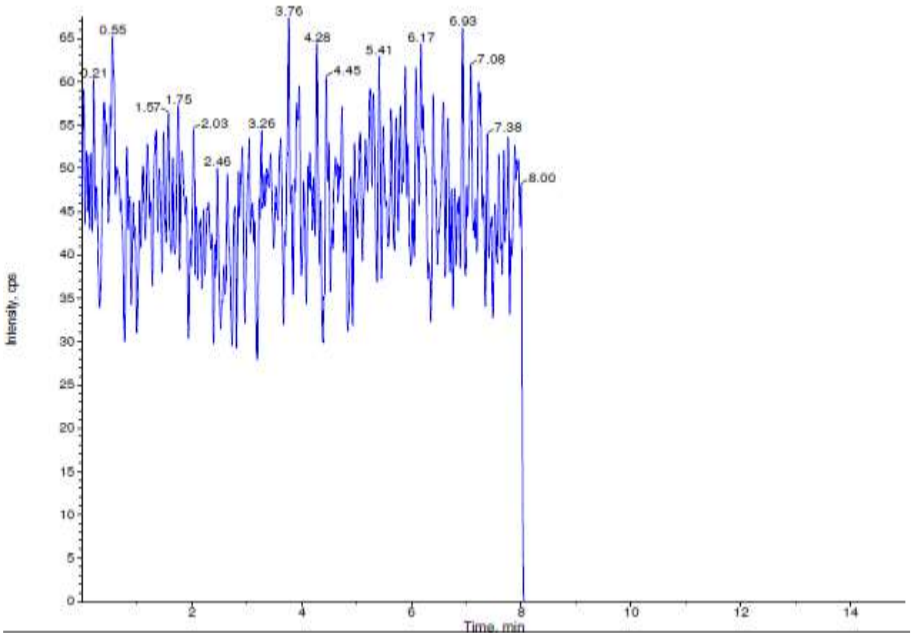
Initially X-Bridge column C18 (150 mm length, 4.6 mm ID & 3.5 µm particle magnitude) and X-Select CSH column C18 (100 mm length, 3.0 mm ID & 2.5 µm particle magnitude) using mobile phase blend of 0.01% ammonia solution with methanol/acetonitrile were tried. When used X-Bridge column C18 and mobile phase blend of 0.1% ammonia solution with acetonitrile, resolution among RC-A and RC-B was <1.5. But with Select CSH column C18 and mobile phase blend of 0.1% ammonia solution with methanol, acceptable resolution (>2.0) was obtained. Consequently, the same was chosen to solve recovery problems owing to the overlap of peaks moving to the mass source along with targeted analytes, resulting in lower level recovery problems. Further improved the gradient mode, flow stream and pH to get better resolution amongst RC-A, RC-B and RLE. Finally, pH optimized was 5.5 (adjusted using formic acid) and the procedure for gradient elution was improved as follows: 55% volume of 0.1% ammonia solution and 45% volume of methanol from 0 - 7 min; 10% volume of ammonia solution and 90% volume of methanol from 7.1 - 10.0 min; 55% volume of ammonia solution and 45% volume of methanol at from 10.1 - 15.0 min. As the concentration of the sample is used further to achieve a sensitivity of 0.75 ppm, the diverter value programme has applied as precautionary measure to prevent contamination of the sample at the mass source, so if the high sample is moved through the mass source due to the deposition of the sample at the source repeatability and recovery problems would occur.

3.2. Validation

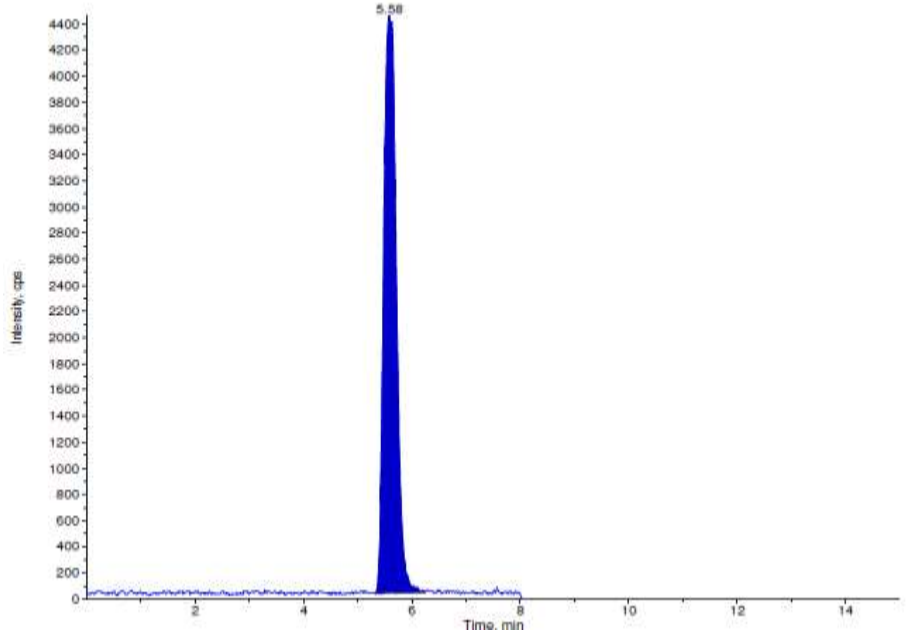
The proposed LC-MS/MS approach was verified according to ICH strategies to determine the reliability, consistency and evenness of the analytical outcomes [17].

3.2.1. Specificity

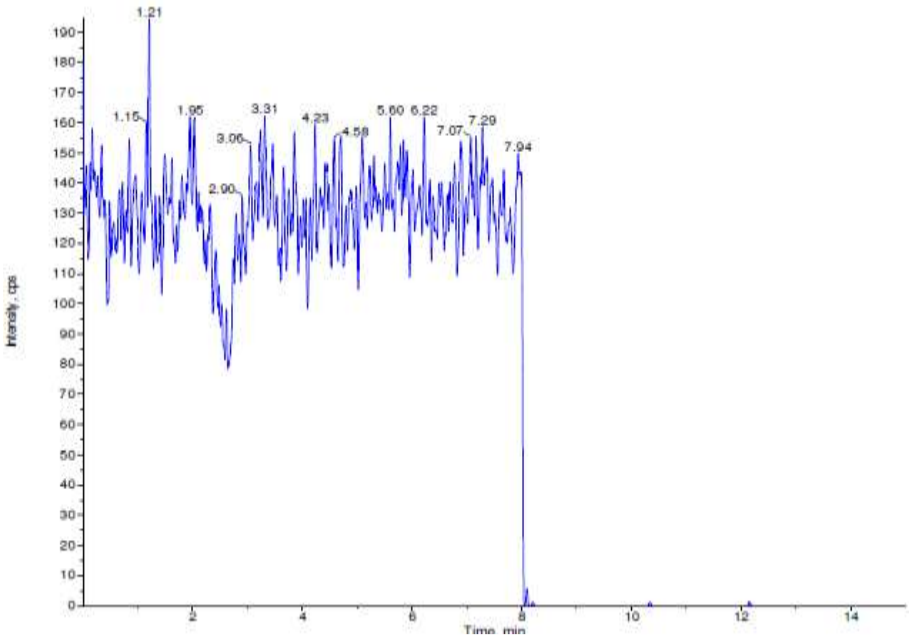
Specificity was appraised by injecting (10 µl) separately the individual working solutions of RC-A (0.75 ppm) and RC-B (0.75 ppm), and diluent blank in to X-Select CSH C18 column and analysed with proposed conditions of LC-MS/MS. Chromatograms are given in Figure 2. No interference was found with diluent component at the RC-A (5.58 min) and RC-B (5.98 min) retention times. This proved specificity of LC-MS/MS approach to analyse RC-A and RC-B.



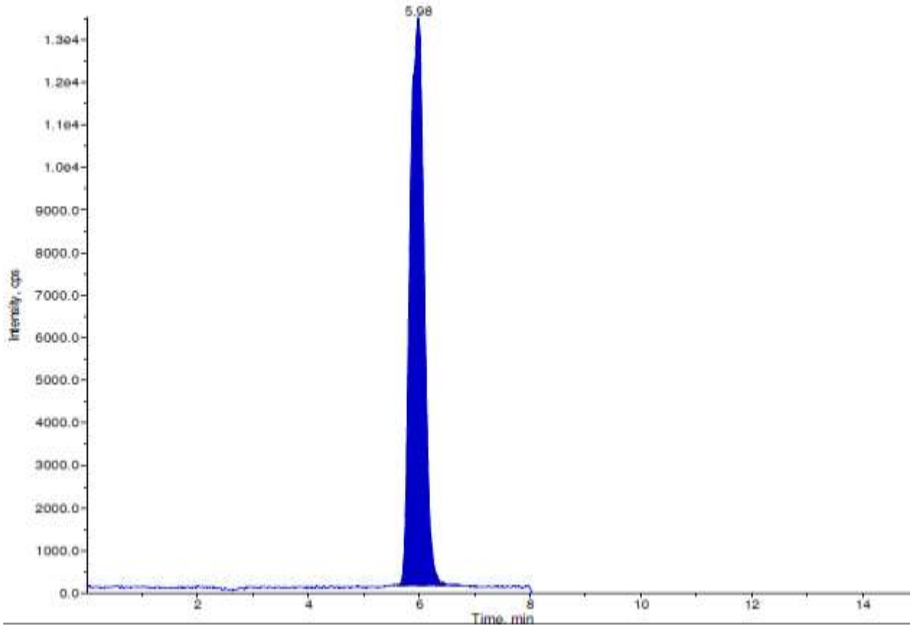
Chromatogram a



Chromatogram b



Chromatogram c



Chromatogram d

Figure 2 Chromatogram a – diluent blank for RC-A; Chromatogram b – working RC-A solution; Chromatogram c - diluent blank for RC-B; Chromatogram d - working RC-B solution

3.2.2. Limit of Quantification (LOQ) and Limit of Detection (LOD)

Diluted the RC-A and RC-B solution quantitatively and stepwise with diluent. The diluted solutions were separately injected into X-Select CSH C18 column and analysed with proposed conditions of LC-MS/MS. LOQ and LOD were described as RC-A and RC-B concentrations (ppm) which could be detected and give signal to noise proportion values of ≥10 and ≥3,

respectively. The LOQ and LOD values (Table 1) evidenced the sensitivity of LC-MS/MS approach to analyse RC-A and RC-B at trace levels.

Table 1 LC-MS/MS methodology sensitivity results

Content	LOQ		LOD	
	S/N Proportion	ppm	S/N Proportion	ppm
RC-A	34.6	0.251	9.9	0.075
RC-B	70.4	0.258	34.6	0.077

S/N - signal to noise

3.2.3. Linearity

Five calibration RC-A and RC-B solutions with RC-A at 0.251 to 1.128 ppm concentration range and RC-B at 0.258 to 1.141 ppm concentration range were analysed thrice. The calibration curves of RC-A and RC-B were developed by mapping the mean area obtained against concentrations. The regression equation and linearity correlation coefficient were calculated for RC-A and RC-B curves (Table 2). The linearity correlation coefficient values (>0.99) evidenced the linearity of LC-MS/MS approach.

Table 2 LC-MS/MS methodology linearity and line equation results

Parameter	RC-A	RC-B
Linearity	0.251 to 1.128 ppm	0.258 to 1.141 ppm
Line equation	A = 217290.1*x*-275.4	A = 672785 *x*-5218.6
Y-Intercept	-275.4	-5218.6
Slope	217290.1	672785
% Y-Intercept	-0.2	-1
Linearity Correlation Coefficient	0.9997	0.9997

A – peak area; *x* – concentration of RC-A/RC-B in ppm

3.2.4. System precision

Separately injected (10 µL) working RC-A and RC-B solution (0.75 ppm) into X-Select CSH C18 column, analysed with proposed conditions of LC-MS/MS in six replicates and noted the peak areas of RC-A and RC-B. The percent RSD between the peak areas of RC-A and RC-B from six replicates were 0.8% and 2.9% respectively (Table 3). This proved system precision to analyse RC-A and RC-B.

3.2.5. Method precision

Separately injected (10 µL) RLE sample spiked with pure RC-A (0.75 ppm) and RC-B (0.75 ppm) into X-Select CSH C18 column and analysed with proposed conditions of LC-MS/MS in six replicates. The concentration of RC-A and RC-B were determined. The percent RSD between the concentrations of RC-A and RC-B from six replicates were 1.0% and 1.3% respectively (Table 3). This proved LC-MS/MS method precision to analyse RC-A and RC-B in RLE sample.

Table 3 LC-MS/MS methodology precision results

Samples	RC-A		RC-B	
	System precision	Method precision	System precision	Method precision
	Peak area	Amount obtained (ppm)	Peak area	Amount obtained (ppm)
1	177717	0.707	582718	0.761
2	178678	0.702	583310	0.745
3	179893	0.707	596208	0.749
4	176095	0.709	547670	0.756
5	176816	0.706	583946	0.741

6	179190	0.69	587772	0.735
Average	178064.833	0.704	580270.667	0.748
SD	1452.238	0.007	16743.997	0.01
RSD	**0.8**	**1.0**	**2.9**	**1.3**

3.2.6. Ruggedness

Ruggedness was appraised by conducting a precision analysis in six prepares of the RLE samples spiked with pure RC-A (0.75 ppm) and RC-B (0.75 ppm) by different analysts (n=2) and on different days (n=2). All of the results disclosed percent RSD not above than 3.0% (2.9% for RC-A and 2.7% for RC-B, Table 4). This proved LC-MS/MS method ruggedness to analyse RC-A and RC-B in RLE sample.

Table 4 LC-MS/MS methodology ruggedness results

Day and analyst	**RC-A amount obtained (ppm)**	**RC-B amount obtained (ppm)**
Day 1 and analyst 1	0.681	0.713
	0.691	0.71
	0.716	0.751
	0.707	0.743
	0.653	0.699
	0.689	0.729
Day 2 and analyst 2	0.681	0.713
	0.691	0.71
	0.716	0.751
	0.707	0.743
	0.653	0.699
	0.689	0.729
Overall Average	0.697	0.736
Overall SD	0.02	0.02
Overall % RSD	2.9	2.7

3.2.7. Recovery

Accuracy was tested by undertaking a recovery analysis in compliance thru ICH guidance. Known concentration of RC-A and RC-B standard solutions corresponding to LOQ level, 100% and150% of specification limit quantity (0.75 ppm) was add up to the RLE sample solution (4000 ppm). The accuracy was measured for these concentrations by three times sample solution injection, and the findings were given in Table 5. As seen (Table 5), the recoveries of RC-A and RC-B were found as94.1%–106.0% and 96.2% - 99.2%, respectively which proved LC-MS/MS method accuracy in analysing RC-A and RC-B in RLE sample.

Table 5 LC-MS/MS methodology accuracy results

Accuracy level	**Amount Add up (ppm)**	**Amount obtained (ppm)**	**Recovery (%)**	**Average of Recovery (%)**
RC-A recovery				
LOQ	0.25	0.270	108.0	106.0
	0.25	0.280	112.0	
	0.25	0.245	98.0	
100%	0.75	0.708	94.3	94.1
	0.75	0.703	93.6	
	0.75	0.708	94.3	
150%	1.15	1.143	99.4	99.0
	1.15	1.140	99.1	
	1.15	1.133	98.5	
RC-B recovery				
LOQ	0.25	0.249	99.6	99.2

	0.25	0.261	104.4	
	0.25	0.234	93.6	
100%	0.75	0.754	97.4	96.2
	0.75	0.738	95.4	
	0.75	0.742	95.9	
150%	1.15	1.114	96.9	97.0
	1.15	1.119	97.3	
	1.15	1.115	97.0	

3.2.8. Robustness

The robustness had been tested by modifying column oven temperature. Robustness was appraised by analysis in three prepares of the RLE samples spiked with pure RC-A (0.75 ppm) and RC-B (0.75 ppm) with modified and optimized column oven temperature. The percent relative difference for the mean content of RC-A and RC-B observed from the results obtained with modified and optimized column oven temperature were 0.0% - 2.8% and 1.7% - 1.8%, respectively (Table 6). This proved LC-MS/MS method robustness for the variation studied in analysing RC-A and RC-B in RLE sample.

Table 6 LC-MS/MS methodology robustness results

Temperature	RC-A		RC-B	
	Mean amount obtained* (ppm)	**Relative difference (%)**	**Mean amount obtained* (ppm)**	**Relative difference (%)**
43 ºC	0.954	0.0	0.768	1.8
45 ºC	0.954		0.754	
47 ºC	0.954	2.8	0.768	1.7
45 ºC	0.981		0.781	

* mean of three values

3.2.9. Solution stability

The stability of RC-A and RC-B solution was judged by analysing the working RC-A and RC-B solution at 0.75 ppm concentration. The stability of the RC-A and RC-B in stored (room temperature) stock solution during 24 hr and 48 hr was determined by comparing it with the fresh stock RC-A and RC-B solution. The percent relative difference for the content of RC-A and RC-B between results obtained in initial and at predetermined intervals were given Table 7. The values proved stability of RC-A and RC-B in solution upto 48 hr.

Table 7 Stability of RC-A and RC-B in solution

Time	RC-A		RC-B	
	Mean amount obtained* (ppm)	**Relative difference (%)**	**Mean amount obtained* (ppm)**	**Relative difference (%)**
0 hr	0.954	-5.3	0.768	-3.1
24 hr	1.005		0.792	
0 hr	0.954	3.8	0.768	6.9
48 hr	0.918		0.717	

* mean of three values

3.2.10. System suitability

Suitability testing is commonly executed to determine the adequacy and efficiency of the whole LC-MS/MS system during the RC-A and RC-B analysis period. System suitability was tested by calculating the correlation between two working solutions (0.75 ppm) of RC-A and RC-B, and percent relative standard deviations of RC-A and RC-B peak areas obtained from six replicate injections of one working solution (0.75 ppm). As revealed in Table 8, correlation factors were in range of 0.97 - 1.30 and percent relative standard deviations were in range of 0.5% - 8.7% for RC-A and RC-B. The values proved suitability of LC-MS/MS system for analysis of RC-A and RC-B.

Table 8 LC-MS/MS device suitability results

Parameter	RC-A Peak area in		RC-B Peak area in	
	WS 1	WS 2	WS 1	WS 2
Test done before specificity evaluation				
Average*	83853	82881	283834	280560
SD	1411.158	2874.417	3999.685	9399.731
%RSD	1.7	3.5	1.4	3.4
Correlation factor	1.30		0.98	
Test done before linearity evaluation				
Average*	179635	177092	541656	535776
SD	2337.232	7059.901	11225.683	18630.219
%RSD	1.3	4.0	2.1	3.5
Correlation factor	0.97		0.97	
Test done before precision evaluation				
Average*	178064.833	178129	580270.667	584642.571
SD	1452.238	1336.532	16743.997	19168.448
%RSD	0.8	0.8	2.9	3.3
Correlation factor	1.30		1.30	
Test done before ruggedness evaluation				
Average*	152117.667	150482	436071.333	435019.143
SD	8909.598	9212.959	38029.188	34827.178
%RSD	5.9	6.1	8.7	8.0
Correlation factor	1.02		0.99	
Test done before accuracy evaluation				
Average*	157890	156870	500726.167	497807.571
SD	6543.31	6554.324	34863.573	32749.321
%RSD	4.1	4.2	7.0	6.6
Correlation factor	0.99		0.99	
Test done before robustness evaluation				
Average*	188260.167	188039.143	653173.167	651019
SD	934.597	1034.337	8787.635	9840.485
%RSD	0.5	0.6	1.3	1.5
Correlation factor	1.00		1.00	
Test done before solution stability evaluation				
Average*	157889.667	157932.714	500726.167	498846.571
SD	6543.31	5974.283	34863.573	32212.122
%RSD	4.1	3.8	7.0	6.5
Correlation factor	0.99		0.99	

3.3. Application of LC-MS/MS methodology developed

The LC-MS/MS methodology developed and validated was applied to detect and quantify RC-A and RC-B in six batches of RLE samples. The results are provided in Table 9. The RC-B content was below detection limit (0.075 ppm) in all batches of RLE while RC-A content was below quantification limit (0.250 ppm) in all batches of RLE except two batches A19CL 2224 and A19CL 2225.

Table 9 Batch analysis of RC-A and RC-B

Batch number of RLE sample	RC-A content quantified (ppm)	RC-B content quantified (ppm)
A19CL2220	BQL	BDL
A19CL2221	BQL	BDL
A19CL2222	BQL	BDL
A19CL2223	BQL	BDL
A19CL2224	0.361	BDL

A19CL2225	0.392	BDL
A19CL2226	BQL	BDL

BQL – below quantification limit; BDL - Below detection limit

4. Conclusion

The developed LC-MS/MS methodology provides accurate, sensitive, specific and precise analysis for the concurrent quantitation of two impurities, RC-A and RC-B, in RLE samples. This method had the benefits of reduced LD and LQ values for RC-A and RC-B. This LC-MS/MS methodology has been shown to be effective for quality evaluation of the RLE sample for RC-A and RC-B impurities.

Author contribution

Arthanareeswari. M, SRM Institute of Science and Technology, Kattankulathur, Department of Chemistry, Tamilnadu, 603203, India. Ravi Uppala, Vijayasri Organics Limited, Analytical Research, Hyderabad 500 072, India. Designed the experiment; Arthanareeswari. M, SRM Institute of Science and Technology, Kattankulathur, Department of Chemistry, Tamilnadu, 603203, India. Ravi Uppala, Vijayasri Organics Limited, Analytical Research, Hyderabad 500 072, India. Carried them Out; Arthanareeswari. M, SRM Institute of Science and Technology, Kattankulathur, Department of Chemistry, Tamilnadu, 603203, India. Ravi Uppala, Vijayasri Organics Limited, Analytical Research, Hyderabad 500 072, India. Venkatasubbaiah. B, Dr Reddys laboratories limited, Analytical Research, Hyderabad 500 072, India. Kishore Gaddam, Hetero R&D, Analytical Research, Hyderabad 500 018, India. Prepared the manuscript with contribution from all co-authors; Arthanareeswari. M, SRM Institute of Science and Technology, Kattankulathur, Department of Chemistry, Tamilnadu, 603203, India. Venkatasubbaiah. B, Dr Reddys laboratories limited, Analytical Research, Hyderabad 500 072, India. Reading of manuscript.

REFERENCES

1. Reddy BM, Weintraub HS, Schwartzbard AZ. Ranolazine: a new approach to treating an old problem. Tex Heart Inst J. 2010;37(6):641-47.
2. Rayner-Hartley E, Sedlak T: Ranolazine: A Contemporary Review. J Am Heart Assoc. 2016;5(3):e003196.
3. Gomberg-Maitland M, Schilz R, Mediratta A, Addetia K, Coslet S, Thomeas V, et al. Phase I safety study of ranolazine in pulmonary arterial hypertension. Pulm Circ. 2015;5(4):691-700.
4. Andrea R, Lucia B, Chiara C, Sara B, Alessia V, Anna D et al. Ranolazine: effects on ischemic heart. Recent Pat Cardiovasc Drug Discov. 2013;8(3):197-203.
5. Bhandari B, Subramanian L. Ranolazine, a partial fatty acid oxidation inhibitor, its potential benefit in angina and other cardiovascular disorders. Recent Pat Cardiovasc Drug Discov. 2007;2(1):35-9.
6. Sampath A, Goverdhan G, Raghupathi RA, Srinivas K, Pratap RP, Prabhakar RV. Improved process for ranolazine: An antianginal agent. Org. Process Res. Dev, 2012;16(5):748–54.
7. Vakamulla M, Anumala RR,Mukkanti K, Suryanarayana MV. A novel reverse phase stability indicating RP-UPLC method for the quantitative determination of fifteen related substances in ranolazine drug substance and drug product. Talanta. 2012;97:563-73.
8. Madhavi A, Rao DVS, Srinivasu P, Naidu A. Development and validation of a new analytical method for the determination of related components and assay of ranolazine in bulk drug and pharmaceutical dosage forms by LC. Chromatographia, 2009; 70(1/2):333–8.
9. Chen Y, Wu S, Yang Q. Development and Validation of LC-MS/MS for Analyzing Potential Genotoxic Impurities in Pantoprazole Starting Materials. J Anal Methods Chem. 2020;2020:6597363.
10. Yenugu VMR, Ambavaram VBR, Moniruzzaman M, Madhavi G. A simple, sensitive, and straightforward LC-MS approach for rapid analysis of three potential genotoxic impurities in rabeprazole formulations. J Sep Sci. 2018;41(21):3966-73.
11. Iliou K, Malenović A, Loukas YL, Dotsikas Y. Analysis of potential genotoxic impurities in rabeprazole active pharmaceutical ingredient via Liquid Chromatography-tandem Mass Spectrometry, following quality-by-design principles for method development. J Pharm Biomed Anal. 2018;149:410-8.
12. Shackman JG. Rapid, direct, and sensitive determination of aziridine and 2-chloroethylamine by hydrophilic interaction liquid chromatography-mass spectrometry. MethodsX. 2019;6:2176-80.
13. Reddy SR, Reddy KH, Kumar MN, Reddy PM, Reddy JVR, Sharma HK. A Validated GC-MS Method for the Determination of Genotoxic Impurities in Divalproex Sodium Drug Substance. J Chromatogr Sci. 2019;57(2):101-7.
14. Liu Z, Fan H, Zhou Y, Qian X, Tu J, Chen B, et al. Development and validation of a sensitive method for alkyl sulfonate genotoxic impurities determination in drug substances using gas chromatography coupled to triple quadrupole mass spectrometry. J Pharm Biomed Anal. 2019;168:23-9.
15. Vinod KA, Rajiv AJ, Vipul PR, Harshal RB, Ravindra DY. Time-dependent selected reaction monitoring-based GC-MS/MS method for estimation of genotoxic impurities in new antibacterial agent: alalevonadifloxacin mesylate. J Anal Sci Technol. 2020;11:article22.
16. Wolff FC, Dillenburg TL, Venzon Antunes M, Linden R, Comparsi Wagner S, Verza SG. Characterization of imatinib mesylate formulations distributed in South American countries: Determination of genotoxic impurities by UHPLC-MS/MS and dissolution profile. Biomed Chromatogr. 2018;32(7):e4222.
17. ICH. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use, ICH Harmonized Tripartite Guideline, ICH Q2A. Text on validation of analytical procedures, Geneva, Switzerland, November 2005.