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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TOLPERISONE AND PARACETAMOL BY RP-HPLC

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ABSTRACT

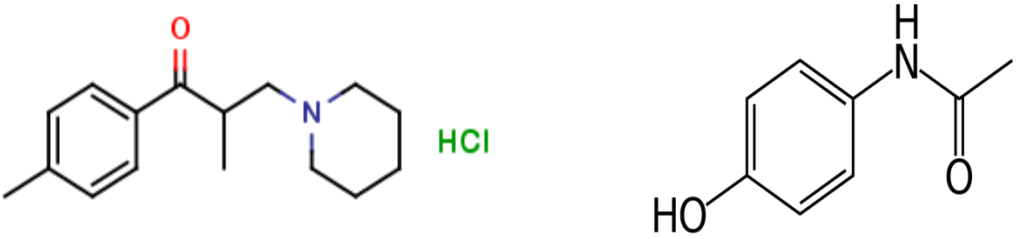
A simple, sensitive, accurate, precise and rapid reverse phase high performance liquid chromatographic method has been developed and validated for the simultaneous determination of Tolperisone and Paracetamol from synthetic mixture. The chromatographic separation was performed on Imo Sil 5 C18 column (250 mm × 4.6 mm i.d, 5 µm particle size). Mobile phase consisted of a Acetonitrile and methanol in the ratio of 25:75, v/v at a flow rate of 1.0 ml/min. The detection wavelength was set at 261nm. The proposed method was validated for linearity, accuracy, precision, LOD and LOQ. The calibration was linear over the concentration range of 10-30 µg/ml for Tolperisone and 5-15 µg/ml for Paracetamol. The retention times were found to be 5.3 ± 0.14min for Paracetamol and 2.4 ± 0.13min for Tolperisone. The mean recoveries were 100.5 ± 0.34 and 98.2 ±0.80 for Tolperisone and Paracetamol, respectively. The method can be easily adopted for quality control analysis.

KEYWORDS: Tolperisone, Paracetamol, HPLC, Validation.

INTRODUCTION

Tolperisone (R,S)2-methyl-1-(4-methyl phenyl)-3-propane-1-one, is a centrally acting muscle relaxant. Acts at reticular formation in the brain stem by inhibiting voltage gated Na^{++}

and Ca^{++} channels. Paracetamol,is N-acetyl-para-aminophenol.acetaminophen, Inhibit the function of COX outside the CNS. Used treat analgesic, antipyretic.



Tolperisone **Paracetamol**

Fig. 1: Tolperisone & Paracetamol.

Tolperisone and Paracetamol (Fig.1) in combination used for the treatment of persistent allergic rhinitis. Literature survey have few analytical methods are available for the simultaneous estimation of Tolperisone and Paracetamol in pharmaceutical formulations by using UV and HPLC. Hence, we made an attempt to develop a simple method for the simultaneous estimation of Tolperisone and Paracetamol by RP-HPLC in pharmaceutical dosage forms.

The present communication describes simple, sensitive, rapid, accurate, precise and cost effective RPHPLC method for simultaneous estimation of both drugs in their combined synthetic mixture. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines.[1-3]

Method Development and Optimization

Development of RP-HPLC method for simultaneous estimation of **Tolperisone and Paracetamol** in combined dosage forms. Shimadzu gradient HPLC system with following configurations was used for the present study such as LC-20AD prominence solvent delivery system (Pump), Rheodyne 7725i injector with 20 µl loop, SPD-20A Prominence UV-VISBLE detector, LC-solution Version 1.25SP4 data station and Analytical column: Imp Sil,C_{18}HS (250 x 4.6 mm i.d., 5µ).

The wavelength of 261 nm was selected for the final method as these drugs has shown good absorbances. The drugs selected in the present study were polar in nature and hence RP-HPLC method was preferred because of its simplicity and suitability.[4-6]

Optimized chromatographic conditions

The following chromatographic conditions were used:

Stationary phase	: Imp Sil,C_{18}HS (250 mm x 4.6 mm i.d, 5µm)
Mobile phase	: Solvent A: Acetonitrile
	Solvent B: Methanol
Solvent ratio	: 25:75
Detection wavelength	: 261 nm
Flow rate	: 1.0 ml/min
Temperature	: Room temperature of 20 ± 2^0 C

Estimation of Tolperisone and Paracetamol

i. Preparation of Standard Solution: 10 mg of Tolperisone was taken in a 10 ml standard flask. To this, mobile was added for dissolving the drug. It was shaken for one min. to obtain a clear solution and the volume was made up to 10 ml with mobile phase. 5 mg of Paracetamol was taken in a 5 ml standard flask and diluted with few ml of mobile phase until the sample dissolves completely and the volume was made up to 5 ml with mobile phase.

ii. Preparation of Formulation Solutions

Twenty tablets were weighed and finely powdered. Powder equivalent to 150 mg of Tolperisone and 325 mg of Paracetamol was accurately weighed into a 100 ml volumetric flask, 30 ml of diluents was added and sonicated for 15 min, made up to the volume with diluents and mixed. Filter the solution through 0.45 nylon membrane filter. Dilute 0.16 ml of the above solution of Tolperisone to 25 ml volumetric flask and diluted to volume with diluents (10µg/ml of Tolperisone). Dilute 0.11ml of the above solution of Paracetamol to 25 ml volumetric flask and diluted to volume with diluents (15µg/ml of Tolperisone). A representative chromatogram of sample preparation (10µg/ml of Tolperisone and 15 µg/ml of Paracetamol).[7-10]

iii. Method of Recording of chromatogram

With the optimized chromatographic conditions mentioned above, a steady baseline at about 10 min. was recorded. After the stabilization of the baseline at about 30 min., the standard solutions were injected and chromatograms were recorded until the reproducibility of the peak areas was satisfactory. Finally 10 µg/ml of the standard solution of Tolperisone and 5 ug/ml of Paracetamol individually were injected and the chromatograms were recorded

(*Fig.2*). Successive aliquots of mixed standard solutions of the calibration curve were injected and the chromatograms were recorded.[11-12]

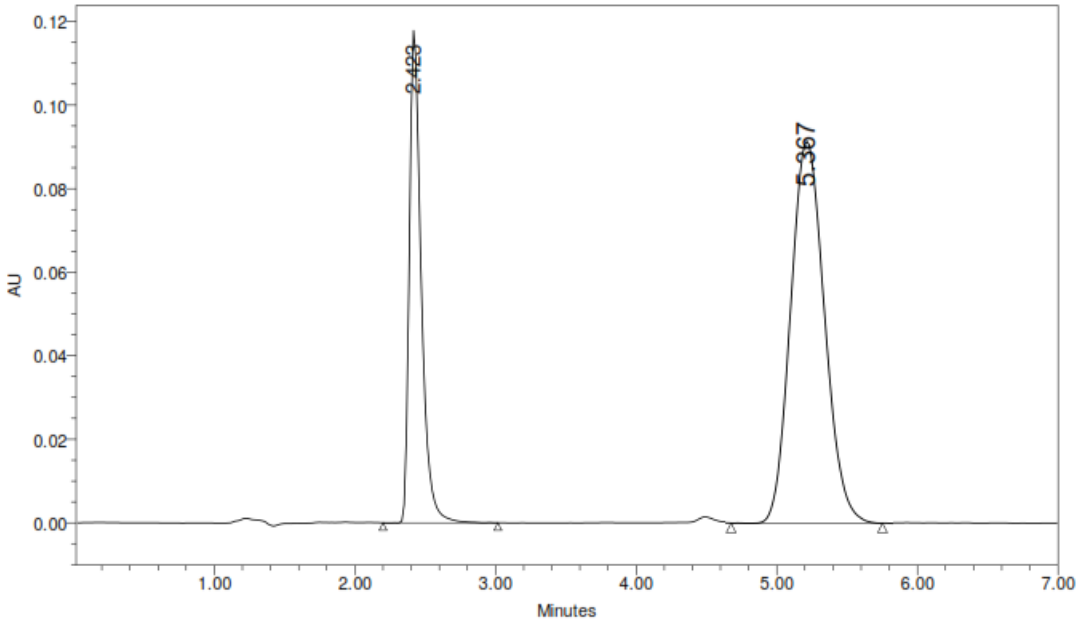


Fig. 2: Chromatogram of Sample solution of brand (Myotop-P).

This procedure was repeated for the sample solution. The peak areas were noted and the response factors of the standard and sample solution peaks were calculated. The elution order of mixture was found as Tolperisone (retention time 2.423 min), and Paracetamol (retention time 5.367 min).

Validation of HPLC Method

After the development of HPLC method for the estimation of multi component dosage form, validation of the method was performed statistically. This section describes the procedure followed for validation of the method developed.

a) Accuracy

Accuracy of the method was determined by recovery experiments. To the formulation, the reference standards of the respective drugs were added at the level of 50% and100 %. These were further diluted by procedure as followed in the estimation of formulation. The concentrations of the drugs present in the resulting sample solution were determined by using assay method.

b) Precision

Repeatability and reproducibility studies demonstrated the precision of the method. Repeatability studies were done by consequently injecting the standard solution at three different concentrations. These solutions were prepared in duplicate and injected as per assay procedure. For the same, Intraday, Interday and reproducibility of injection were studied.

c) Linearity and Range

From the standard stock solutions, a suitably mixed standard solution was prepared to contain 50 to 150% of targeted level of the assay concentration of the standard drugs. The solutions were examined by the assay procedure. The calibration curve (Fig. 3& 4) was plotted using response factor (peak area ratio of the standard peak area Vs concentration of the standard solutions for both the drugs. The values thus obtained were mentioned in Table 7 for Tolperisone and table 8 for Paracetamol from the calibration curve, the slope and intercept are calculated.[13-14]

d) Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of the developed method were determined by analyzing progressively lower concentrations of the standard solution using optimized chromatographic conditions. The minimum concentration of the standard solution, which gave signal to noise ration of 3 and 10 were taken as the LOD and LOQ values respectively. The LOD and LOQ were found by formula given in the ICH guide line for validation.[15-17]

e) System Suitability Studies

The system suitability studies were carried out as specified in USP. These parameters include column efficiency, resolution and capacity factor Table 6.

RESULTS AND DISCUSSION

The peak area ratios of standard and sample solutions were calculated .The assay procedure was repeated for 6 times and mean peak area, mean peak area ratio, mean weight of standard drugs, mean weight of sample taken for assay were calculated. The percentages of individual drugs found in formulations, mean, and standard deviation in formulations were calculated and presented in Table 1. The results of analysis shows that the amount of drugs was in good agreement with the label claim of the formulations.[17]

Table 1: Analysis of Formulation.

Formulation	Drug	Label Claim (mg/tablet)	Estimated Amount* (mg/tablet)	% Label claim*	SD*
(**Myotop-P**)	Tolperisone	150	149.96	99.35	0.12
	Paracetamol	325	324.57	99.86	0.10

* Average of six Determination

2. Validation of the method

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and results are percentage relative standard deviation were calculated and presented in Table 2. Recoveries of standard drugs were found to be accurate and were within the specified limits.

Table 2: Accuracy (Recovery Studies).

Drug name	Label Claim (mg/tablet)	% Amount added	Amount found	% Recovery	% RSD
TOLPERISONE	150	50	200.78	100.78%	0.125
		100	249.24	99.60%	
PARACETAMOL	325	50	374.25	99.73%	1.125
		100	424.30	99.83%	

The precision of the method was determined by studying repeatability and reproducibility. The intraday, Interday studies were carried out and the Peak areas of drug peaks and percentage relative standard deviation were calculated and results are presented in Table. 3 & 4. The results revealed that the method developed is reproducible and its results are shown in Table 5& 6.

Table 3: Intraday Studies.

Level	Concentration (µg/ml)		Peak area		% RSD	
	Tolperisone	Paracetamol	Tolperisone	Paracetamol	Tolperisone	Paracetamol
I	10	5	1458967	1854724	1.256	1.258
			1365789	1852479		
			1425878	1845742		
II	15	7.5	2145879	2657854	0.140	0.456
			2256874	2785241		
			2354780	2657892		
III	20	10	2954178	3524785	0.025	0.124
			2895241	3625478		
			2954782	3547890		

Table 4: Interday Studies.

Day	Concentration (µg/ml)		Peak area		% RSD	
	Tolperisone	Paracetamol	Tolperisone	Paracetamol	Tolperisone	Paracetamol
1	10	5	1425478	1852478	0.245	0.125
2			1352478	1852147		
3			1420145	1812453		
1	15	7.5	2112450	2621456	1.250	0.145
2			2147852	2724521		
3			2214578	2624521		
1	20	10	2925478	3520142	0.123	0.156
2			2820451	3624508		
3			2925410	355421		

The linearity range for Tolperisone was found to be from 10 to 30 µg/ml and the linearity range for Paracetamol was found to be 5-15µg/ml respectively.

Repeatability of injection

A standard solution of mixture of drugs were injected six times and its % RSD was calculated and results shown in table 5**.**

Table 5: Repeatability of injection.

Concentration (µg/ml)	Injection	Peak area		% RSD	
		Tolperisone	Paracetamol	Tolperisone	Paracetamol
Tolperisone (10 µg/ml) Paracetamol (5 µg/ml)	1	145870	1852478	0.0401	0.0230
	2	140273	1852147		
	3	140733	1812453		
	4	140676	182458		
	5	140567	1835689		
	6	140373	1825890		

The response factor, slope, intercept and correlation coefficient values were calculated. The correlation coefficient of Tolperisone and Paracetamol were found to be 0.998 and 0.998 respectively. The calibration curves were plotted using response factor Vs concentration of the standard solutions (Fig. 6 and 7).

LOD of Tolperisone and Paracetamol were found to be 0.196 ug /ml, 0.127. The LOQ of Tolperisone and Paracetamol were found to be 0.327, 0.322 ug /ml. The resolution, capacity factor, theoretical plates/meter, peak symmetry was finding out for the standard solutions and is presented in Table 6.

The values obtained demonstrated the suitability of the system for the analysis of the above drug combination.

Table 6: System suitability studies.

Drug	R_s	N	K'	Tailing factor	HETP	LOD ug/ml	LOQ ug/ml
Tolperisone	2.358	2354.0	0.541	1.25	54.25	0.196	0.327
Paracetamol	9.258	5232.4	2.145	1.02	25.35	0.127	0.322

Rs =Resolution**, N** =Theoretical plate**, K'** =Capacity factor, **LOD** = Limit of detection **LOQ** = Limit of quantification**, HETP** = Height equivalent to therapeutic plates.

Table 7: Linearity of Tolperisone.

Tolperisone		
S.No	**Concentration (µg/ml)**	**Peak area**
1.	10	1400921
2.	15	2105853
3.	20	2952278
4.	25	3625789
5.	30	4305891

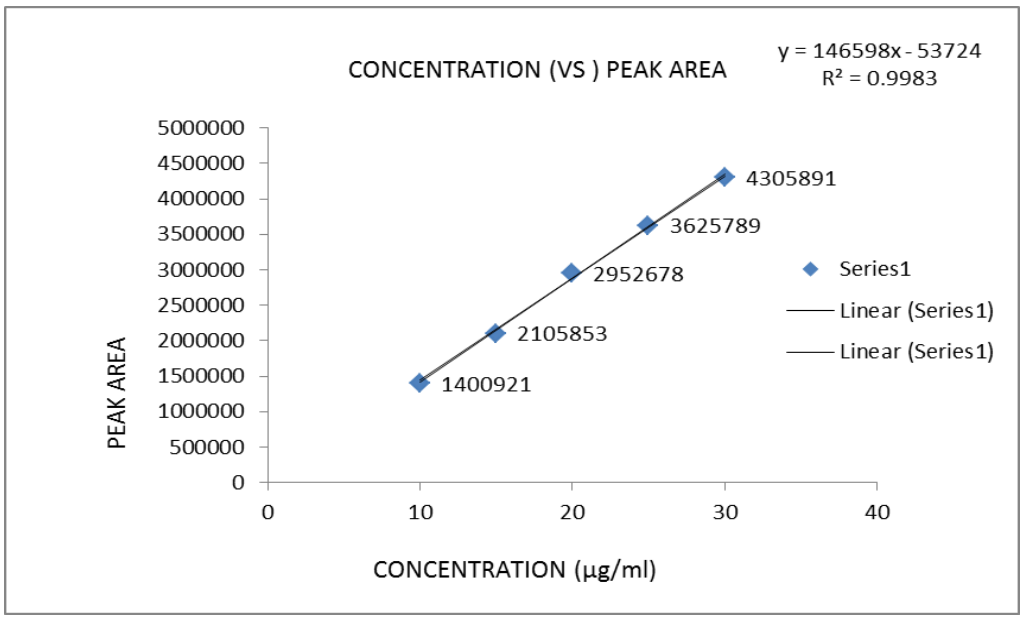


Fig. 3: Calibration curve for Tolperisone.

Table 8: Linearity of Paracetamol.

Paracetamol		
S. No.	**Concentration (µg/ml)**	**Peak area**
1.	5	1823587
2.	7.5	2647892
3.	10	3598745
4.	12.5	4365897
5.	15	5235878

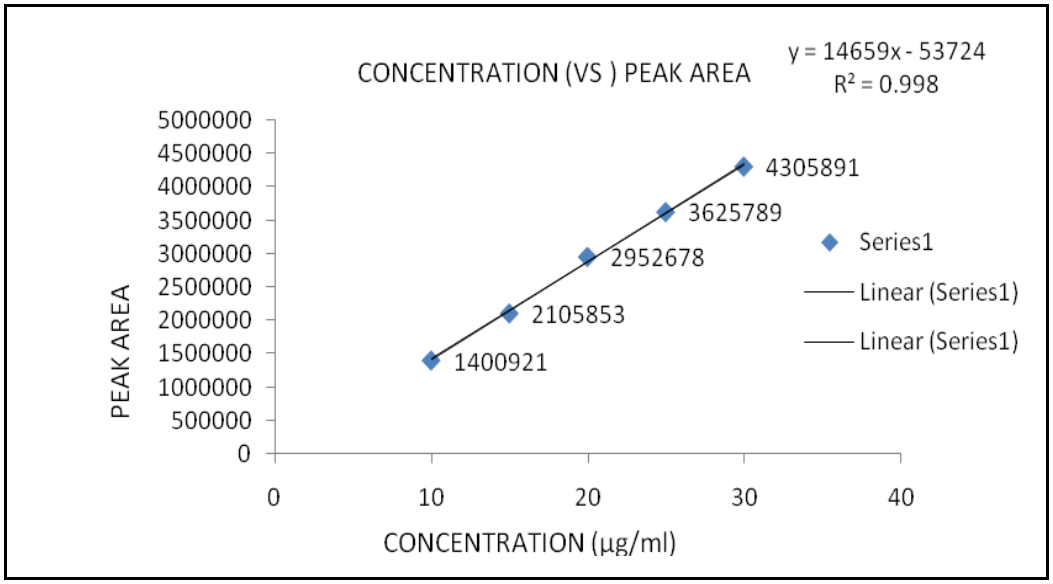


Fig. 4: Calibration curve for Paracetamol.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameter and provides an indication of its reliability during normal usage. As part of the robustness, deliberate change in the flow rate, mobile phase composition, temperature variation was made to evaluate the impact on the method. The typical variations are variation in flow rate by ±0.2 ml/min and variation in temperature varied from ±10%. Mixed samples of both Paracetamol and Tolperisone were analyzed under these changed experimental conditions. The results of robustness study are shown in Table 8.

Table 8: Robustness for Paracetamol and Tolperisone.

S. No.	Parameter	Paracetamol		Tolperisone	
		Peak area	Tailing factor	Peak area	Tailing factor
1.	Flow Rate (+0.2ml/min)	2245279	1.545	2658744	1.124
2.	Flow Rate (-0.2ml/min)	2253214	1.321	2785481	1.254
3.	Temp. change (10% more)	2363780	1.548	2658742	1.321
4.	Temp. change (10% less)	2145879	1.365	2658422	1.987
% RSD		0.58		0.25	

Ruggedness

The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots by different analysts using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The assay was performed

in different condition, different analyst and different dates. The results of ruggedness study are given in Table 9.

Table 9: Ruggedness for Paracetamol and Tolperisone.

S. No.	Parameter	Paracetmol		Tolperisone	
		Peak area	Tailing factor	Peak area	Tailing factor
1.	Analyst-1	2254789	1.524	2598476	1.145
2.	Analyst-2	2245874	1.214	2845762	1.245
3.	Analyst-3	2354721	1.219	2658789	1.231
4.	Analyst-4	2123654	1.321	2652314	1.210
% RSD		0.120		0.021	

CONCLUSION

The deliberate changes in the method and operational conditions have not much affected the chromatograms. This indicates that the proposed method was robust and rugged. The proposed RP-HPLC method was simple, specific, sensitive, precise and accurate and can be used for simultaneous estimation of Tolperisone and Paracetamol in bulk samples and its tablet dosage forms.

REFERENCES

1. Murali, M., & Satyanarayana, P. V. (2011). Simple validated isocratic RP–HPLC method for estimation of Tolperisone in bulk and pharmaceutical dosage form. *Der Pharma Chemica*, *3*(5): 13-19.
2. Bae, J. W., Park, Y. S., Sohn, U. D., Myung, C. S., Ryu, B. K., Jang, C. G., & Lee, S. Y. (2006). HPLC determination of tolperisone in human plasma. *Archives of pharmacal research*, *29*(4): 339-342.
3. Koladia, B. B., & Vaghela, V. M. (2012). UV spectroscopic method for quantitative estimation of tolperisone hydrochloride in bulk and pharmaceutical dosage form. *Int J PharmTech Res*, *4*: 1317-22.
4. Nimila, I. C., Balan, P., Chiranjeevi, N., Maheswari, V. U., & Rajasekhar, S. (2011). Method development and statistical validation of UV spectrophotometric method for tolperisone hydrochloride in bulk and tablet dosage form. *J Pharm res*, *4*: 1356-1357.
5. Liawruangrath, S., Liawruangrath, B., & Pibool, P. (2001). Simultaneous determination of tolperisone and lidocaine by high performance liquid chromatography. *Journal of pharmaceutical and biomedical analysis*, *26*(5-6): 865-872.
6. Patel, M. J., Badmanaban, R., & Patel, C. N. (2011). Reversed phase-high performance liquid chromatographic method for simultaneous estimation of tolperisone hydrochloride

and etodolac in a combined fixed dose oral formulations. *Pharmaceutical methods*, *2*(2): 124-129.

7. Patel, S. A., & Hariyani, K. P. (2012). Spectrophotometric method for simultaneous estimation of tolperisone hydroc.
8. Patel, M., Shah, R., Kadikar, H., Patani, P., & Shukla, M. (2012). Method development and statistical validation of UV spectrophotometric method for estimation of Tolperisone hydrochloride and Paracetamol in synthetic mixture and combined dosage form. *Int J Pharm Res Bio Sci*, *1*(1): 1-19.hloride and diclofenac sodium in synthetic mixture.
9. Raju, T. V. R., Seshadri, R. K., Arutla, S., Mohan, T. S. S. J., Rao, I. M., & Nittala, S. R. (2012). Development and validation of a precise, single HPLC method for the determination of Tolperisone impurities in API and pharmaceutical dosage forms. *Scientia pharmaceutica*, *81*(1): 123-138.
10. Sharma, K. K., & Patel, P. U. (2012). First Derivative Spectrophotometric Method for the Simultaneous Estimation of Tolperisone and Paracetamol in their Combined Dosage Form. *Journal of pharmaceutical science and bioscientific research*, *2*(2): 92-96.
11. Patel, M. J., Badmanaban, R., & Patel, C. N. (2011). RP-HPLC method for simultaneous estimation of Tolperisone hydrochloride and Etodolac in combined fixed dosage oral formulation. *Pharm methods*, *2*: 124-129.
12. Srinivas, G., Vidyadhara, S., Ramanaiah, G., & Srilakshmi, V. (2014). Method development and validation of stability indicating RP-HPLC method for simultaneous estimation of thiocolchikoside and aceclofenac in bulk and its pharmaceutical formulations. *International Journal of Bioassays*, *3*(4): 2059-2065.
13. Abirami, G., & Vetrichelvan, T. (2013). Simultaneous determination of Tolperisone and Paracetamol in pure and fixed dose combination by UV-Spectrophotometry. *Inter. J. Pharm. Pharm Sci*, *5*: 488-492.
14. Patel, D. S., Captain, A. D., Prajapati, P. P., & Shah, H. G. (2013). Development and validation of HPTLC method for simultaneous determination of tolperisone hydrochloride and diclofenac sodium in combined dosage form. *Int J Pharm Tech Res*, *5:* 147-54.
15. 15. Narapusetti, A., Bethanabhatla, S. S., Sockalingam, A., Repaka, N., & Saritha, V. (2015). Simultaneous determination of rosuvastatin and amlodipine in human plasma using tandem mass spectrometry: Application to disposition kinetics. *Journal of advanced research*, *6*(6): 931-940.

16. Narapusetti, A., Bethanabhatla, S. S., Sockalingam, A., & Pilli, N. R. (2015). LC-MS/MS assay for Acetazolamide, A Carbonic Anhydrase Inhibitor in Human Plasma and its Clinical Application. *Journal of Young Pharmacists*, *7*(4): 438.
17. Narapusetti, A., Bethanabhatla, S. S., Sockalingam, A., Pilli, N. R., Repaka, N., & Alla, T. (2014). Bioanalysis of riluzole in human plasma by a sensitive LC-MS/MS method and its application to a pharmacokinetic study in South Indian subjects. *Analytical Methods*, *6*(13): 4823-4830.