Novel UV Spectrophotometer Methods for Quantitative Estimation of Empagliflozin (EMPA) and *Linagliptin* (Lina) Using Mixed Hydrotropy Solubilization

Udai Bhan Singh Rathore [a*] and Pradeep Kumar Goyal [a]

[a] *B. N. College of Pharmacy, Bhupal Nobles' University, Udaipur-313001 (RAJ.), India.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Simple, precise accurate, novel and safe UV-Spectrophotometric simultaneous equation method developed for the simultaneous estimation of poorly water-soluble drugs Empagliflozin (EMPA) and Linagliptin (LINA) in tablet dosage form using 2M ammonium acetate: 2M sodium citrate and (50:50% W/W) as mixed hydrotropic solution and validated as per ICH guidelines. This Method involves solving of simultaneous equations based on measurement of absorbance at two wavelengths 270 nm and 294 nm (λ_{max} of EMPA and LINA) in hydrotropic solutions. Ammonium acetate and sodium citrate solution did not show any absorbance above 240 nm and thus no interference in the estimation of drugs was seen. EMPA and LINA follow Beers law in the concentration range of 10-50μg/ml and 5-25μg/ml (r2 = 0.999). % Recovery for both the drugs was in the range of 98.50 to99.23 % indicating excellent accuracy. The methods were precise, with a relative standard deviation of less than 2% for both drugs. The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. Thus, method can be used for routine monitoring of drugs in industry for the assay of bulk drugs and commercial formulation.

1. INTRODUCTION

Empagliflozin (EMPA) is used as a sodium glucose cotransporter-2 (SGLT-2) inhibitor to improve glycemic control in adult patients with type 2 diabetes. SGLT-2 co-transporters reabsorb glucose from the glomerular filtrate in kidney and the glucuretic action resulting from inhibition of SGLT-2 which reduces renal absorption and lowers down the renal threshold for glucose, therefore increases glucose excretion which reduces hyperglycaemia and also helps in blood pressure reduction [1, 2]. Chemically EMPA is 1-chloro-4-(glucopyranos-1-yl)-2-(4-(tetrahydrofuran-3-yloxy)benzyl)benzene and having empirical formula is $C_{23}H_{27}ClO_7$ with molecular weight 450.91 g/mole (Fig. 1A). Linagliptin (LINA) is having competitive, reversible DPP-4 inhibitory action which is responsible for DPP-4 breakdown reduction of GLP-1 and glucose-dependant insulin tropic polypeptide (GIP). From beta cells of the pancreas, GLP-1 and GIP stimulate the release of insulin during inhibiting release of glucagon from pancreatic beta cells. These effects together reduce the breakdown of glycogen in the liver and increase insulin release in response to glucose [2-4]. Chemically LINA is (R)-8-(3-aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methylquinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione and having empirical formula is $C_{25}H_{28}N_8O_2$ with molecular weight 472.5422 g/mole (Fig. 1B). Literature review revealed that few methods were described for the determination of EMPA and LINA alone or in combination with other drugs from pharmaceutical dosage forms and in human plasma including spectrophotometry [5-8], ultra-performance liquid chromatography (LC) [9], LC–mass spectroscopy [10], and high-performance LC (HPLC) [11-17] techniques. Hydrotropic solubilization is the phenomenon by which aqueous solubility of poorly water soluble drugs and insoluble drugs increases. Various techniques have been employed to enhance the aqueous solubility and hydrotropy is one of them. Sodium salicylate, sodium benzoate, urea, nicotinamide, sodium citrate and sodium acetate are the most common examples of hydrotropic agents utilized to increase the water solubility of the drug. Maheshwari and Jain et al. have analyzed various poorly water-soluble drugs using hydrotropic solubilization phenomenon viz. ketoprofen, salicylic acid, frusemide, torsemide, hydrochlorothiazide, pramipexole and amlodipine besylate [18-26]. Various organic solvents such as methanol, chloroform, dimethyl formamide and acetonitrile have been employed for solubilization of poorly water-soluble drugs to carry out spectrophotometric analysis. Drawbacks of organic solvents include their higher cost, toxicity and pollution. Hydrotropic solution may be a proper choice to preclude the use of organic solvents. Therefore, it was thought worthwhile to employ this mixed hydrotropic solution to extract out the drug from fine powder of tablets to carry out spectrophotometric estimation. There are no reports yet for the determination of this combination by proposed methods. The present work emphasizes on the quantitative estimation of EMPA and LINA in their combined dosage form by UV Spectroscopic methods.
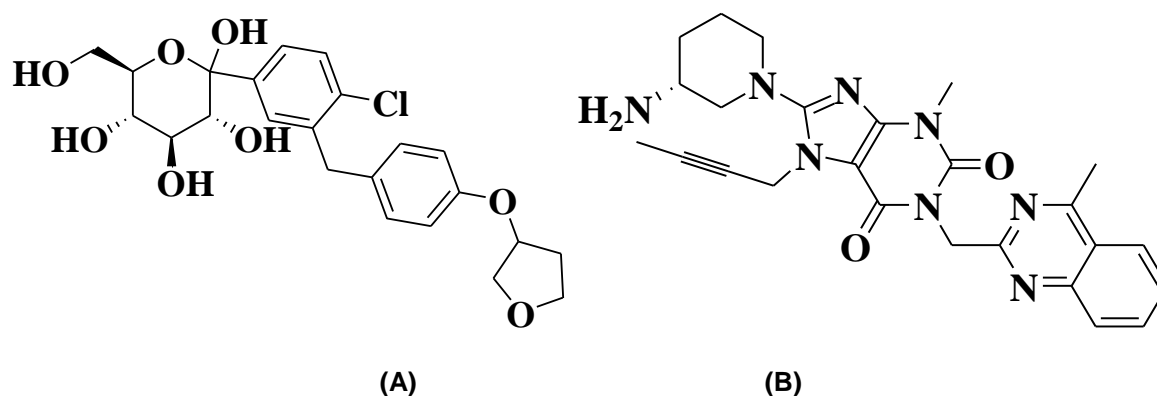

Fig. 1. Chemical structure of (A) Empagliflozin and (B) Linagliptin

2. MATERIALS AND METHODS

2.1 Experimental Procedure

EMPA and LINA standard were obtained from Hetero drugs Ltd, Hyderabad, India. Methanol, acetonitrile were procured from Rankem, RFCL Limited, New Delhi, India. Ammonium acetate AR, sodium citrate and sodium benzoate AR grade, etc were procured from Central Drug House (P) Limited, New Delhi, India. The 0.45-mm pump nylon filter was obtained from Advanced Micro devices (Ambala Cantt, India). Reverse osmosis water was used throughout the study. Other chemicals used were of analytical or HPLC grade. Glyxambi Tab (10mg/5mg) was purchased from local market.

2.2 Preliminary Solubility Studies of Drugs

Solubility of both drugs was determined at 25 ± 1^0C. An excess amount of drug was added to two screw capped 25 ml of volumetric flasks containing different aqueous systems viz. distilled water and different combination of hydrotropic agent. The volumetric flasks were shaken mechanically for 12 h at 25 ± 1^0C in a mechanical shaker. These solutions were allowed to equilibrate for next 24 h. and then centrifuged for 5 min at 2000 rpm. The supernatant liquid was taken for appropriate dilution after filtering through Whatman filter paper #41 and analyzed spectrophotometrically against water as blank. After analysis, it was found that the enhancement in the solubility of EMPA and LINA was found to be more than 60 to 70% in a mixture of 2 M ammonium acetate: 2M sodium citrate solution (1:1) as compared to solubility studies in other solvents.

2.3 Establishment of Stability Profile

Stability of EMPA and LINA was observed by dissolving in a mixture of 2M ammonium acetate: 2M sodium citrate (50:50 % V/V) solution used as hydrotropic agent. Solution of EMPA and LINA was scanned under time scan for 30 min. Spectra of the drug under time scan shows that drug is stable in hydrotropic solution.

2.4 Linearity Range and Calibration Graph

2.4.1 Preparation of standard stock solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80`ml mixed hydrotropic solution containing 2M ammonium acetate: 2M sodium citrate (1:1) and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to 100 ml with mixed hydrotropic agent to get a concentration of 1000 µg/ml (Stock-A) for both drugs.

2.4.2 Preparation of sub stock solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of EMPA and LINA and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with RO Water that gave concentration of 100 µg/ml (Stock-B).

2.4.3 Preparation of working standard solution

- Aliquots of 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml withdrawn with help of pipette from standard stock solution (Stock-B) separately in 10 ml volumetric flask and volume was made up to 10 ml with RO Water. This gave the solutions of 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml respectively for EMPA.
- 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with RO Water. This gave the solutions of 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml and 25 µg/ml respectively for LINA.

2.5 Selection of Wavelength for Linearity

Solutions of 5µg/ml of EMPA and 10µg/ml LINA were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of EMPA and LINA was observed at 270.0 nm and 294.0 nm, respectively. EMPA and LINA showed linearity in the concentration range of 10-50µg/ml and 5-25µg/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration Figs. 2, 3.

2.6 Study of Overlay Spectra

Working standard solution from the standard stock solution prepared in concentration 5µg/ml of EMPA and 10 µg/ml of LINA were scanned in the spectrum mode over the range of 200-400 nm against RO Water as blank and the overlain spectra of the two were

recorded. EMPA showed an absorbance peak at 270.0 nm, whereas LINA at 294.0 nm. The overlain spectra also showed isoabsorptive points at 280.0 nm. Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method Fig. 4.
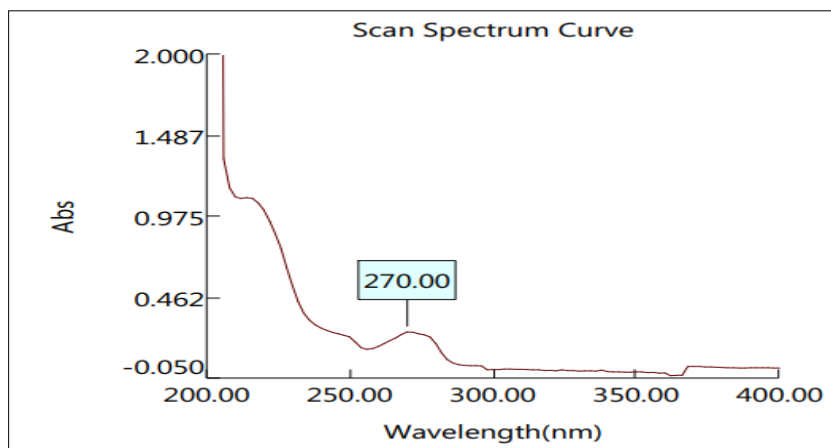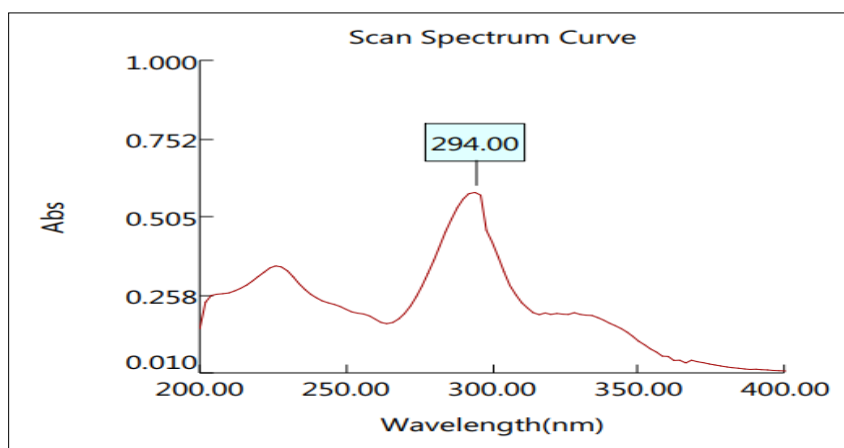

Fig. 2. Determination of λ_{max} of EMPA

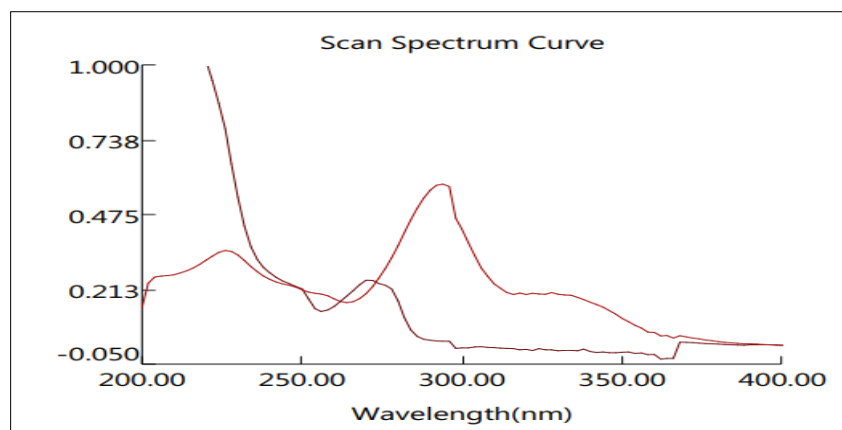**Fig. 3. Determination of λ_{max} of LINA**

Fig. 4. Overlay spectra of EMPA and LINA

2.7 Vierordt's Simultaneous Equation Method

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 270.0 nm and 294.0 nm that are λ_{max} of EMPA and LINA respectively. The absorbance was measured at the selected wavelengths and absorptivities ($A^{1\%, 1cm}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

$$C\ EMPA = \frac{A1ay2 - A2ay2}{ax1ay2 - ax2ay1} \ldots \ldots \ldots \ldots \ldots \ldots . Eq. (1)$$

$$C\ EMPA = \frac{A1ax2 - A2ax2}{ax1ay2 - ax2ay1} \ldots \ldots \ldots \ldots \ldots \ldots . Eq. (2)$$

Where, A_1 and A_2 are absorbance of mixture at 270 nm and 294 nm respectively, ax_1 and ax_2 are absorptivities of EMPA at λ_1 (270.0 i.e. λ_{max} of EMPA) and λ_2 (294.0 i.e. λ_{max} of LINA) respectively and ay_1 and ay_2 are absorptivities of LINA at λ_1 and λ_2 respectively. C_{LINA} and C_{EMPA} are concentrations of EMPA and LINA respectively. Figure represent the overlain spectra of both the drugs in 1:2 ratio and the criteria for obtaining maximum precision [i.e. absorbance ratio $(A_2/A_1)/ax_2/ax_1$ and ay_2/ay_1] by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the EMPA and LINA [27].

2.8 Methods Validation

Validation of the method was carried out in accordance with the International Conference on Harmonization Q2B guidelines 2005 [28].

2.8.1 Linearity

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and estimate into the UV and the results was recorded. The results of linearity are reported in Table 1.

2.8.2 Accuracy

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in Table 2.

2.8.3 Precision

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and % RSD are less than 2 indicate the precision of method. Result of precision shown in Table 3.

2.8.4 Analysis of tablet sample

Twenty marketed tablets of EMPA and LINA were weighed and ground to a fine powder; amount equal to 10mg of EMPA was taken in 10 ml volumetric flask. The LINA present in this amount of tablet powder was 5mg. Then 8 ml of 2M ammonium acetate: 2M sodium citrate (1:1) solution was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with RO Water to get the final concentrations of both drugs in the working range. The absorbance of final dilutions was observed at selected wavelengths and the concentrations were obtained from simultaneous equation method. The procedure was repeated for five times Table 4.

Table 1. Result of linearity of empagliflozin (EMPA) and linagliptin (LINA)

	Method	
Parameter	EMPA	LINA
Working λ	270 nm	294 nm
Beer's law limit (µg/ml)	10-50	5-25
Correlation Coefficient (r^2)*	0.998	0.999
Slope (m)*	0.014	0.022
Intercept (c)*	0.003	0.003

value of five replicate

Table 2. Results of recovery studies

Recovery level %	% Recovery (Mean±SD)*	
	EMPA	LINA
80	99.23±0.464	98.50±0.667
100	99.17±0.334	98.59±0.486
120	99.09±0.343	98.90±0.949

*Average of three determination

Table 3. Results of precision

Parameter		Method	
		(Mean±SD)*	
		EMPA	LINA
Precision*	Repeatability	99.221±0.106	98.354±0.106
	Day-to-Day	99.592±0.015	99.205±0.015
	Analyst-to-Analyst	99.235±0.151	99.245±0.058
	Reproducibility	99.504±0.114	99.666±0.039

*Average of five determination

Table 4. Analysis of tablet formulation of EMPA and LINA

Drug	Label claim (mg)	Amount found (mg)	Label claim (%)	S.D.	% RSD
EMPA	10	9.95	99.50	0.115	0.135
LINA	5	4.92	98.40	0.142	0.148

3. RESULTS AND DISCUSSION

Based on the solubility and stability and spectral characteristics of the drugs, 2M ammonium acetate: 2M sodium citrate solutions (50:50% W/V) were used as a mixed hydrotropic solution. It was found that solubility enhancement of EMPA and LINA was more than 60 to70%, respectively in mixed hydrotropic solution as compared with distilled water. EMPA and LINA show maximum absorbance at 270 nm and 294 nm, respectively. Ammonium acetate and sodium citrate did not show any absorbance above 240 nm and thus no interference in the estimation of drugs was seen. EMPA and LINA follow Beer's law in the concentration range of 10-50µg/ml and 5-25µg/ml ($r2$ =0.999 and 0.999). Simultaneous equation method employed 270 and 294 nm as two analytical wavelengths for estimation of EMPA and LINA. The optimized methods showed good reproducibility and mean recovery with 99.504±0.114, 99.666±0.039 and99.23±0.464, 98.90±0.949 for EMPA and LINA, respectively. The mean percent label claims of tablet dosage were found to be 99.50 and 98.40 for EMPA and LINA, respectively. The standard deviation, coefficient of variance and standard error were obtained for EMPA and LINA were satisfactorily low. Result of precision at different levels was found to be within acceptable limits (RSD <2).

4. CONCLUSION

There was no interference of 2M ammonium acetate: 2M sodium citrate solution (50:50% W/V) in the estimation and hence the Vierordt's simultaneous equation UV spectrophotometric methods were found to be simple, accurate, economic and rapid for simultaneous estimation of EMPA and LINA in bulk and tablet dosage forms. The proposed method can be successfully employed for the routine analysis of EMPA and LINA containing dosage forms.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Drug bank, Empagliflozin. Available:https:// www. drugb ank. ca/ drugs/ DB090 38
2. Pubchem, Empagliflozin. Available:https:// pubch em. ncbi. nlm. nih. gov/ compo und/ Empag liflo zin
3. Drug bank, Linagliptin. Available:https:// www. drugb ank. ca/ drugs/ DB088 82
4. Pubchem, Linagliptin. Available:https:// pubch em. ncbi. nlm. nih. gov/ compo und/ Linag liptin
5. Banik S, Karmakar P, Miah MA. Development and validation of a UV-spectrophotometric method for determination of vildagliptin and linagliptin in bulk and pharmaceutical dosage forms. Bangladesh Pharm J. 2015;18:163-8.
6. Sangeetha RK, Subashri T. Analysis of linagliptin in tablet dosage form by UV spectroscopy method, its derivatives and difference spectra. Euro J Pharm Med Res. 2016;3:536-40.
7. Padmaja N, Veerabhadram G. Development and validation of analytical method for simultaneous estimation of empagliflozin and linagliptin in bulk drugs and combined dosage forms using UV-visible spectroscopy. Pharm Lett. 2015;7:306-12.
8. Bassam MA. Development and validation of simple spectrophotometric and chemometric methods for simultaneous determination of empagliflozin and metformin: Applied to the recently approved pharmaceutical formulation. Spectrochim Acta Part A. 2016;168:118-22.
9. Ayoub BM. UPLC simultaneous determination of empagliflozin, linagliptin and metformin. RSC Adv. 2015;16:95703-9.
10. Maha FA, Omar AA, Miriam FA, Mariam MT. Pharmaceutical analysis of linagliptin and empagliflozin using LC-MS/MS. Pharma Chem. 2016;8:186-9.
11. Madhusudhan P, Radhakrishna MR, Devanna N. RP-HPLC method development and validation for simultaneous determination of linagliptin and empagliflozin in tablet dosage form. Int Adv Res J Sci Eng Technol. 2015;2:95-9.
12. Donepudi S, Achanta S, validated HPLC-UV method for simultaneous estimation of linagliptin and empagliflozin in human plasma. Int J Appl Pharm. 2018;10:56-61.
13. Padmaja N, Veerabhadram G. Development and validation of a novel stability-indicating RP-HPLC method for the determination of empagliflozin in bulk and pharmaceutical dosage form. Int J Pharm Sci Res. 2016;7:4523-30.
14. Godasu SK, Sreenivas SA. A new validated RP-HPLC method for the determination of metformin HCl and empagliflozin in bulk and pharmaceutical dosage and forms. Int J Pharm Sci Res. 2017;8:2223-32.
15. Patil SD, Amurutkar SV, Chatpalliwar VA, Upasani CD. Development and validation of RP-HPLC method for empagliflozin and metformin HCL. J Innov Pharm Biol Sci. 2017;4:185-9.
16. Afzal SJ, Asif M, Khan PM. Validation of stability indicating high performance liquid chromatographic method for simultaneous determination of assay of linagliptin and metformin drugs in the pharmaceuticals tablet formulations using bupropion as a common internal standard. J Innov Pharm Biol Sci. 2018;5:21-8.
17. Bakshi A, Mounika A, Bhutada S, Raju MB. Simultaneous estimation of empagliflozin and linagliptin by RP-HPLC method. World J Pharm Pharm Sci. 2018;7:1062-71.
18. Maheshwari RK. Analysis of frusemide by application of hydrotropic solubilization phenomenon. Ind. Pharm. 2005;4:55–58.
19. Maheshwari RK. A novel application of hydrotropic solubilization in the analysis of bulk samples of ketoprofen and salicylic acid. Asian J. Chem. 2006;18:393–396.
20. Jain N, Jain R, Jain D, Jain A. Spectrophotometric quantitative estimation of amlodipine besylate in bulk drug and their dosage forms by using hydrotropic agent. Eur. J. Chem. 2010;5:212–217.
21. Jain N, Jain R, Thakur N, Gupta BP, Banweer J, Jain S. Novel spectrophotometric quantitative estimation of torsemide in tablets using mixed hydrotropic agent. Der. Pharm. Lett. 2010;2 :249–254.

22. Ruchi Jain, Nilesh Jain, Deepak Kumar Jain, Vijay Kumar Patel, Harish Rajak, Surendra Kumar Jain. Novel UV spectrophotometer methods for quantitative estimation of metronidazole and furazolidone using mixed hydrotropy solubilization. Arabian Journal of Chemistry. 2017;10:151–156

23. Jain N, Jain R, Jain DK, Maheshwari RK, Jain SK: Novel UV-Spectrophotometric Method for Quantitative Estimation Of Furazolidone Using Mixed Hydrotropic Agent, Pak. J. Pharm. Sci. 2013; 26(1):159-162.

24. Jain R, Jain V, Jain N, Jain DK, Jain SK: Eco Friendly Spectrophotometric Method for Quantitative Estimation of Lomefloxacin Using Hydrotropic Approach. Journal of Applied Pharmaceutical Science. 2012; 02(04):111-114.

25. Jain N, Jain R, Kulkarni S, Jain DK, Jain SK. Ecofriendly spectrophotometric method development and their validation for quantitative estimation of Pramipexole Dihyrochloride using mixed hydrotropic agent, J Chem Pharm Res. 2011;3(1):548-552.

26. Jain N, Jain R, Sharma HK, Jain DK, Jain SK: Application of Mixed Hydrotropic Solubilization Phenomenon for Quantitative Analysis of Olmesartan Medoxamil in Tablet The Pharma Review; 2011.

27. Beckett AH, Stanlake JB. Practical Pharmaceutical Chemistry, fourth ed., part 2. CBS Publishers and Distributors, New Delhi; 1997.

28. ICH Guidelines: Validation of Analytical Procedures: Text and Methodology Q2 (B); 2005.