Journal of Pharmaceutical Research International

33(36A): 91-98, 2021; Article no.JPRI.70410
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Simultaneous Determination and Validation of Flupirtine Maleate and Paracetamol in Combined Dosage Form by Chromatographic Technique

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i36A31930
Editor(s):
(1) Dr. Syed A. A. Rizvi, Nova Southeastern University, USA.
Reviewers:
(1) Hua Jiang, Henan University of Science and Technology, China.
(2) Mohammad Anas Suliman, Aligarh Muslim University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/70410

Original Research Article

Received 02 May 2021
Accepted 05 July 2021
Published 09 July 2021

ABSTRACT

Objective: The focus of this research was to establish a validated high-performance thin layer chromatographic (HPTLC) method for analysing Flupirtine maleate and Paracetamol in a combined dosage form.
Method: Paracetamol and Flupirtine maleate were measured using a mobile phase of Ethyl acetate: Chloroform (7:5 v/v) at 286 nm. This technique was validated in accordance with the International Conference on Harmonization (ICH) guidelines.
Results: The Rf value for paracetamol was 0.31 and 0.52 for Flupirtine maleate in this existing technique. Paracetamol's linearity was found to be in the range of 3250-6500 ng/band, while Flupirtine maleate's linearity was found to be in the range of 1000-2000 ng/band. The method's accuracy was determined by recovery experiments, which revealed a percent recovery of 98 to 102 percent. The % RSD was determined to be less than 2 in the Precision investigation, and the assay result for both compounds was within the limit.

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Keywords: Flupirtine maleate; paracetamol; HPTLC; lupirtin-P.

1. INTRODUCTION

Flupiritine maleate is a non opoid, centrally acting analgesic used to treat acute and chronic pain. Ethyl-2-amino-6(4-fluorobenzylamino)3-pyridylcabamate maleate is the chemical name for flupirtine maleate (Fig. 1A). It prevents neuronal excitation by functioning as N-methyl-D-aspartate (NMDA) antagonist [1-3]. As an analgesic and antipyretic, paracetamol is commonly used. Chemically Paracetamol, N-(4-hydroxyphenyl) acetamide (Fig. 1B) effectively blocks the cyclooxygenase enzyme, which is associated for prostaglandin formation [4-6].

Various analytical methodologies using spectrophotometry, HPLC, and stability studies have been mentioned for Flupirtine maleate alone and in combination with other compounds, according to a review of the literature [7-10]. For paracetamol with various combinations, analytical methods such as spectrophotometry, HPLC, UPLC, LC-MS, and stability studies have been published [11-19]. For the determination of Flupirtine maleate and Paracetamol in mixed dosage form, numerous spectrophotometric, chromatographic and stability indicating approaches have been identified in the literature [20-25]. A HPTLC method for estimating Flupirtine maleate and Paracetamol in mixed dosage form is provided in the current work. The proposed approach has been validated in accordance with ICH criteria [26].

2. MATERIALS AND METHODS

2.1 Materials

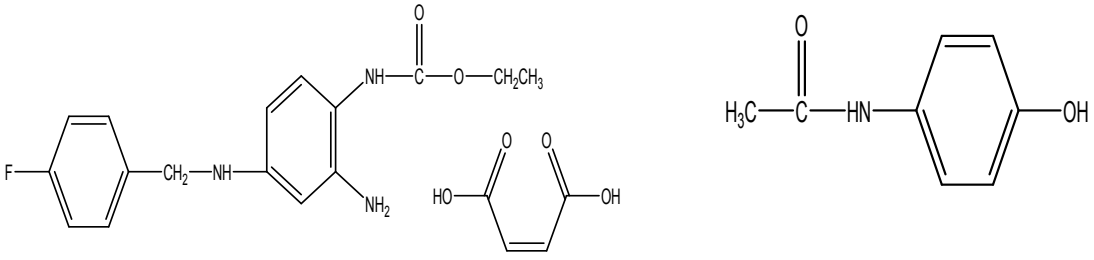
Reference standard of Flupirtine maleate was obtained from Datt Chemicals, Gujarat and Paracetamol was obtained from Yarrow Chem Laboratories, Maharashtra. The marketed formulation of Flupirtine maleate and Paracetamol in combination (Lupirtin-P) was procured from local pharmacy. Methanol, Ethyl acetate, Chloroform and distilled water were used throughout the study are AR grade.

2.2 Chromatographic Conditions

The HPTLC system consist of TLC Aluminum Sheet precoated with Silica Gel 60 F_{254} as stationary Phase, Win- CATS software, 100 µl Hamilton syringe with CAMAG Linomat 5 applicator and 286 nm was selected as the wavelength for measurement of Paracetamol and Flupirtine maleate. On the basis of optimum resolution and R_f value, the mobile phase was selected as a mixture containing Ethyl acetate: Chloroform (7:5 v/v).

2.3 Preparation of Standard Stock Solution of Flupirtine Maleate and Paracetamol

Accurately weighed quantity of Flupirtine maleate and Paracetamol (50 mg) was taken in a 50 ml volumetric flask separately, dissolved and volume made up to mark with methanol (1000 µg/ml). From the stock solutions 1 ml of Flupirtine maleate and 3.25 ml of Paracetamol were taken in a 10 ml volumetric flask, mixed and volume made up with methanol to get a solution containing 100 µg/ml of Flupirtine maleate and 325 µg/ml of Paracetamol, respectively. Each microlitre (µl) of resulting solution contains 100 ng of Flupirtine maleate and 325 ng of Paracetamol.



A **B**

Fig. 1. (A) Chemical structure of A. Flupirtine maleate and B. Paracetamol

2.4 Selection of Wavelength

Standard stock solutions of Paracetamol (3250 ng/band) and Flupirtine maleate (1000 ng/band) were prepared and applied on the precoated TLC plate and scanned using CAMAG HPTLC scanner III. It was observed that Paracetamol and Flupirtine maleate showed considerable absorbance at 286 nm. So, 286 nm was selected as the wavelength for measurement of Paracetamol and Flupirtine maleate throughout the method.

2.5 Preparation of Calibration Curve

Different concentrations of standard Flupirtine maleate solution ranging from 1000-2000 ng/band and Paracetamol solution ranging from 3250-6500 ng/band were prepared by taken 10, 12, 14, 16, 18 and 20 µl from mixed standard stock solution, applied to the plate for the calibration curve of these drugs. Peak area of the spots was measured at 286 nm in the absorbance mode with CAMAG TLC scanner.

2.6 Method Validation [26-28]

2.6.1 Linearity and range

The linearity of response was determined in concentration range of 1000-2000 ng/band for Flupirtine maleate and 3250-6500 for ng/band Paracetamol. The calibration curve was plotted using peak areas vs. concentrations to get correlation-coefficient and regression line equations for Flupirtine maleate and Paracetamol. Linearity is expressed in terms of correlation co-efficient of linear regression line.

2.6.2 Precision

2.6.2.1 *Repeatability*

Mixed standard solution containing Flupirtine maleate (1600 ng/band) and Paracetamol (5200 ng/band) was applied six times, scanned, peak area was measured and % RSD was calculated.

2.6.2.2 Intra-day precision

Variation of results within same day is called Intra-day precision. The Intra-day precision was determined for standard solution of Flupirtine maleate and Paracetamol for the three different concentrations three times on the same day. Peak areas was measured and % RSD was calculated.

2.6.2.3 Inter-day precision

The Inter day precision was determined for standard solution of Flupirtine maleate and Paracetamol for the three different concentrations were analyzed 3 times on the three different days. The % RSD was calculated.

2.6.3 Accuracy

1600 ng/band and 5200 ng/band drug solution of Flupirtine maleate and Paracetamol was taken in three different flask labelled as A, B and C respectively. Spiked 80%, 100%, 120% of standards solution in it and diluted up to 10 ml. The peak area of each solution was measured at 286 nm. The amount of Flupirtine maleate and Paracetamol was calculated at each level and % recoveries were computed.

2.6.4 Limit of detection and limit of quantification

In order to determine the detection and quantification limits, following equations designated by International Conference of Harmonization (ICH) guidelines.

$$LOD = 3.3 \times (SD / Slope)$$
$$LOQ = 10 \times (SD / Slope)$$

2.6.5 Estimation of flupirtine maleate and paracetamol in formulation

20 Tablets crushed and powdered. Powder equivalent to 50 mg of Flupirtine Maleate and 162.5 mg of Paracetamol was taken in a 50 ml volumetric flask, volume made up with methanol and sonicated for 30 minutes, filtered the solution, which contains 1000 µg/ml of Flupirtine maleate and 3250 µg/ml of Paracetamol. From the solution 1 ml was taken in a 10 ml volumetric flask and volume made up to mark with methanol, to get 100 µg/ml of Flupirtine maleate and 325 µg/ml of Paracetamol. The solution was injected 16 µl. The areas of resulting peak were measured at 286 nm.

3. RESULTS AND DISCUSSION

3.1 Mobile Phase Optimisation

Ethyl acetate: Chloroform (7:5 v/v) was selected as mobile phase for the separation of Paracetamol and Flupirtine maleate after tried different solvent system. The Rf values are found

to be 0.31 for Paracetamol and 0.52 for Flupirtine maleate.

3.2 Method Validation

3.2.1 Linearity and range

Flupirtine maleate linearity was observed in the concentration range of 1000-2000 ng/band, while Paracetamol linearity was found in the range of 3250-6500 ng/band (Table 1). Flupirtine maleate and Paracetamol obtained correlation coefficients of 0.996 and 0.994, respectively, for the calibration curve. Fig. 3 depicts the calibration curve for Flupirtine maleate and Paracetamol. The regression line equation was Y=0.864x+2623 for Flupirtine maleate and Y=1.049x+8071 for Paracetamol. Fig. 4 shows the linearity chromatograms of Flupirtine maleate and Paracetamol.

3.2.2 Precision

The repeatability, interday precision and intraday precision of the proposed approach were all evaluated. In Repeatability study same concentration analyzed six times. In intraday precision, the method was analyzed three times on the same day and in interday precision, the method was analyzed three times on the different days. The results shown that the percentage RSD is less than 2% at each level, clearly indicates that the proposed method is precise enough for the analysis of drug (Table 2).

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

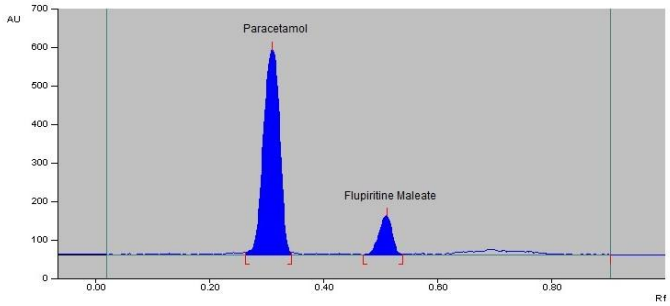
In this proposed method the LOD and LOQ were determined to be 300.38 and 910.24 for Flupirtine maleate, 1042.90 and 3160.32 for Paracetamol respectively (Table 2).

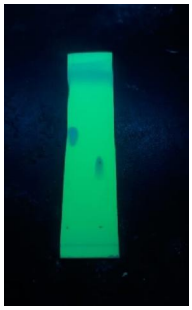
3.2.4 Accuracy

Accuracy of the analytical procedure was established by percentage recovery study from marketed formulation at three level of standard addition. Values of recovery in the range of 98 – 102 % indicate that proposed method is accurate for the analysis of drug (Table 2).

3.3 Estimation of Drug Content in Formulation

The proposed method was used to estimate the drug content in commercially available formulation. The results are shown Table 3. The chromatograms were shown in Fig. 5.





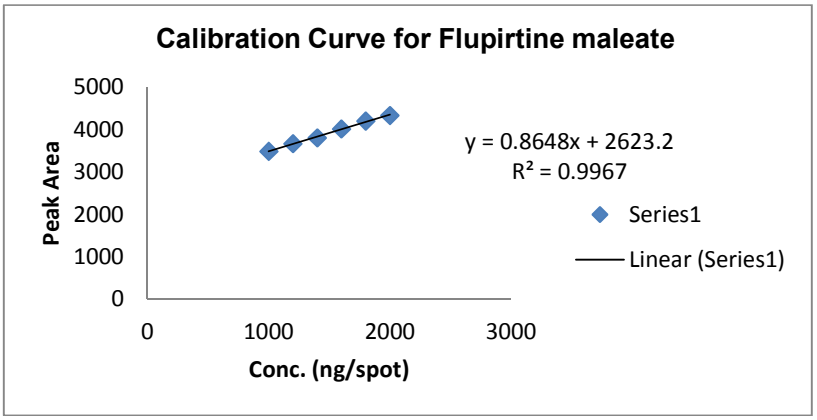
A **B**

Fig. 2. A. Chromatogram of Paracetamol (Rf 0.31) and Flupirtine maleate (Rf 0.52) B. Image of HPTLC plate under UV chamber

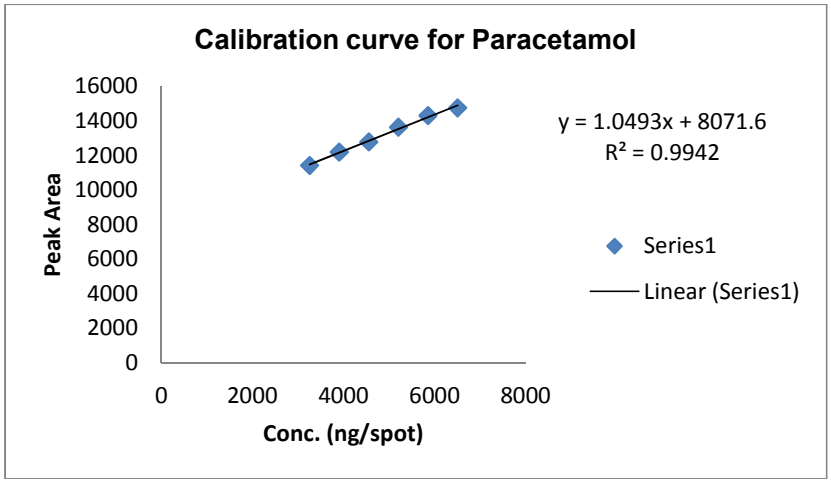
Table 1. Calibration data for Flupirtine maleate and Paracetamol

Flupirtine maleate		Paracetamol	
Conc. (ng/spot)	**Peak area (n=3)**	**Conc. (ng/spot)**	**Peak area (n=3)**
1000	3487.78	3250	11424.75
1200	3669.91	3900	12198.69
1400	3807.57	4550	12786.39
1600	4017.08	5200	13640.07
1800	4203.72	5850	14309.40
2000	4336.33	6500	14761.85

n= number of determinations



(A)



(B)

Fig. 3. Calibration curve of (A) Flupirtine maleate and (B) Paracetamol

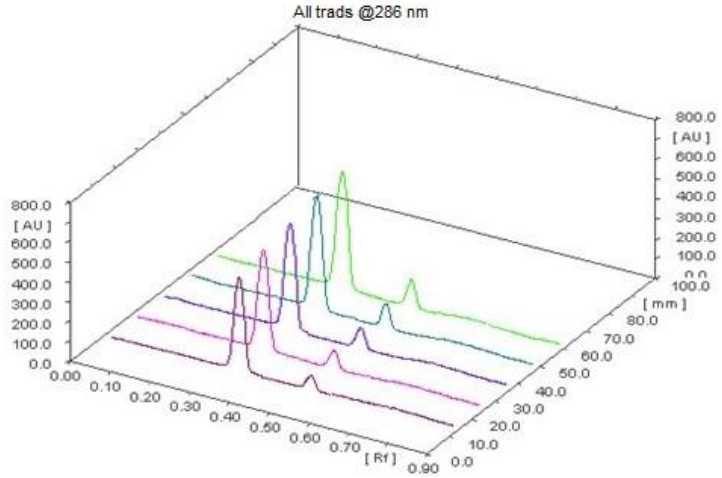


Fig. 4. 3D linearity chromatogram of Paracetamol (Rf: 0.31) and Flupirtine maleate (Rf: 0.52)

Table 2. Summary of Validation Parameters for the HPTLC method

Parameters	Flupirtine maleate	Paracetamol
Linearity Range (ng/spot)	1000-2000	3250-6500
Correlation coefficient	0.996	0.994
Regression equation	Y=0.864x+2623	Y=1.049x+8071
LOD (ng/spot)	300.38	1042.90
LOQ (ng/spot)	910.24	3160.32
Precision (%RSD)		
Repeatability (n=6)	0.02293	0.0055
Intraday (n=3)	0.7995	0.7999
Interday (n=3)	0.9110	0.6890
Accuracy		
80% (n=3)	101.06	99.07
100% (n=3)	100.52	100.31
120% (n=3)	100.16	99.70

n= number of determinations

Table 3. Assay of marketed formulation by proposed HPTLC method

Drugs	Labeled amount (mg)	Amount found (mg)	Amount found (%)*	% RSD
Flupirtine maleate	100	99.30	99.30 ± 0.832	0.837
Paracetamol	325	321.29	98.86 ± 1.323	1.338

** mean ± SD*

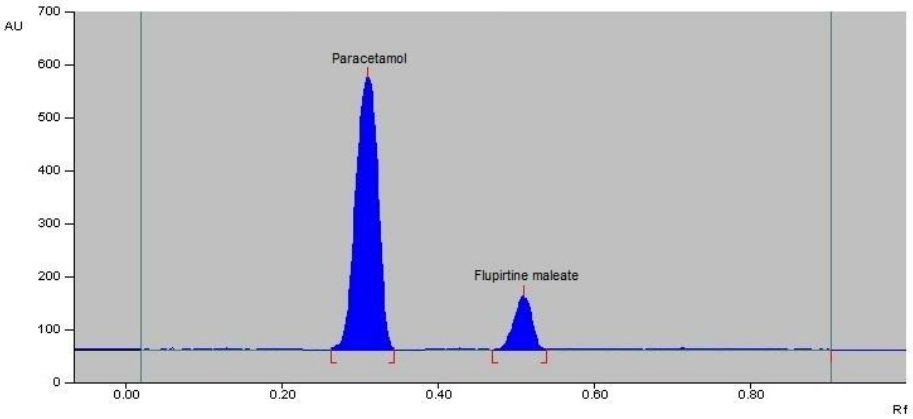


Fig. 5. Chromatograms of Flupirtine maleate and paracetamol in formulation

4. CONCLUSION

For the determination of Flupirtine maleate and Paracetamol in combined dosage form, the suggested HPTLC approach provides a unique quantitative analysis. In compliance with ICH criteria, the suggested method was validated and applied for the determination of Flupirtine maleate and Paracetamol in combine dosage form. For both analytes, the formulation analysis demonstrated good agreement (98-102% w/w) with the label claim. Furthermore, the approach is versatile and effective because to its wider linearity range, lower LOD and LOQ values, lower standard deviation and acceptable percent RSD. Hence the method can be utilized for the simultaneous determination of these two drugs in combined dosage form.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors are thankful to Bhavin Babariya for his technical support to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. The Merck Index, An encyclopedia of chemicals, drug, and biological, 13th edition, White House Station, NJ, Merck & Co., Inc. 2001;742.
2. Martindale, The complete drug reference, 36th edition, Volume I, London, UK, pharmaceutical press (an imprint of RPS publishing). 2009;61.
3. Singal R, Gupta P, Nidhi JA, Gupta S. Role of flupirtine in the treatment of painchemistry and its effects, Maedica. 2012;7(2):163-165.
4. Martindale. The complete drug reference, 36th edition, Volume I, London, UK, pharmaceutical press (an imprint of RPS publishing). 2009;108-111.
5. Indian Pharmacopoeia. Government of India, ministry of health & family welfare, Volume-2, Ghaziabad, Indian Pharmacopoeia Commission. 2007;1514-1517.
6. British Pharmacopoeia, Volume 3, London, UK, stationary office, MHRA. 2008;2965-2970.
7. Aneesh TP, Amal D. Method development and validation for the estimation of flupirtine maleate in bulk and pharmaceutical dosage form by UV spectrophotometry. Int Res J Pharm. 2011;2(12):179-82.
8. Gowrisankar D, Rao Nm. Development and validation of stability indicating UV and RP-HPLC method for the estimation of flupritine maleate in bulk and formulations. Orient J Chem. 2014;30(4): 1933-40.
9. Peraman R, Lalitha KV, Routhu HB. Identification of degradation products and a stability-indicating RP-HPLC method for the determination of flupirtine maleate in pharmaceutical dosage forms. Sci Pharm. 2014;82(2):281-94.
10. Kathirvel S, Sujatha R, Pandit MR, Suneetha A. Stress degradation studies on flupirtine maleate using stability-indicating RP-HPLC method. Chromatogr Res Int. 2013;2013:1-6.
11. Sawant R, Joshi R, Bhangale P. Simultaneous estimation & validation of paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate in tablets by spectrophotometric method. J Pharm Res Health Care. 2011;3(2):23-8.
12. Abdelaleem EA, Abdelwahab NS. Validated stability indicating RP-HPLC method for determination of paracetamol, methocarbamol and their related substances. Anal. Methods. 2013;5(2): 541-5
13. Rajput M, Hamid H, Aggarwal M, Khandal RK. Development and validation of a stability indicating reverse phase HPLC method for simultaneous determination of etodolac and paracetamol in its tablet dosage formulation development. International Journal of Innovative Research in Science, Engineering and Technology. 2015;4(1):19074-86.
14. Devi TP, Setti A, Srikanth S, Nallapeta S, Pawar SC, Rao JV. Method development and validation of paracetamol drug by RP-HPLC. J Med Allied Sci. 2013;3(1): 8-14.
15. Youssef SH, Mohamed D, Hegazy MA, Badawey A. Analytical methods for the determination of paracetamol, pseudoephedrine and brompheniramine in Comtrex tablets. BMC Chem. 2019;13(78):1-15.
16. Kachave RN, Varungase AT, Mandlik PB, Wakchaure SR. Bioanalytical method development and validation for simultaneous determination of paracetamol, tramadol HCL, domperidone tablet formulation by RP-HPLC: Its pharmacokinetic applications. J Pharm Anal. 2018;7(1):6-11.
17. Saranya CL, Thejaswini JC, Gurupadayya BM, Sruthi BY. Simultaneous determination of cefotaxime sodium and paracetamol by LC-MS. IOSR J Pharm. 2014;4(2):12-18.
18. Jena B, Babu S, Pradhan D, Swain S. UPLC analytical method development and validation for the simultaneous estimation of paracetamol and caffeine capsules dosages form. Pharm Regul Aff. 2017;6(186):2-9.
19. Khan H, Ali M, Ahmad S, Ahmad N, Ahuja A, Baboota S et al. Validated UPLC/Q-TOF-MS method for simultaneous

determination of aceclofenac, paracetamol and their degradation products in tablets. J Liq Chromatogr Relat Technol. 2012; 35(1):109-28.

20. Giriraj P, Sivakkumar T. New simple spectrophotometric method for the simultaneous estimation of paracetamol and flupirtine maleate in pure and pharmaceutical dosage form. Int J Spectrosc. 2014;2014:1-6.
21. Shah U, Kavad M, Raval M. Development and validation of UV spectrophotometric method for estimation of paracetamol and flupirtine maleate in bulk and pharmaceutical dosage form. Int J PharmTech Res. 2013;5(3):1007-13.
22. Haritha P, Rao BS, Sunandamma Y. Method development and validation for simultaneous determination of flupirtine maleate and paracetamol by RP-HPLC technique. Int J Pharm Sci Res. 2014;5(2):463-72.
23. Pappula N, Chintala P. Development and validation of RP-HPLC method for the simultaneous estimation of paracetamol and flupirtine maleate in pharmaceutical dosage form. Indian J Pharm Educ Res. 2014;48:34-9.
24. Gullipalli G, Nayeem N, Ranganath MK. Analytical method development and validation for the simultaneous estimation of flupirtine maleate and paracetamol in bulk and pharmaceutical dosage form using RP-HPLC. Int. J. Pharm. Sci. Invent. 2014;3(5):49-59.
25. Nagasarapu M, Dananna G. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of paracetamol and flupirtine maleate in pure and pharmaceutical dosage forms. J Young Pharm. 2015;7(2):81-88.
26. International conference on harmonization (ICH), validation of analytical procedures: Text and methodology Q2(R1), Geneva; 2005.
27. Sen AK, Hinsu D, Sen D, Zanwar A, Maheshwari R, Chandrakar V. Analytical method development and validation for simultaneous estimation of Teneligliptin hydrobromide hydrate and Metformin hydrochloride from it's pharmaceutical dosage form by three different UV spectrophotometric methods. J. Appl. Pharm. Sci. 2016;6(09):157-65.
28. Dash DK, Sen AK, Seth AK, Saisivam S, Sadhu P. Comparison between RP-HPLC and HPTLC techniques for the analysis of ethionamide. Int J Pharm Res. 2020; 12(1):592-7.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/70410