Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 670-675
Received: 16-09-2019
Accepted: 20-10-2019

Aarushi Singh
School of Biotechnology, SKUAST-J, Jammu and Kashmir, India

Vishaw Vikas
Division of Soil Science&Agril. Chem., SKUAST-J, Jammu and Kashmir, India

Narinder Panotra
Organic Farming Research Centre, SKUAST-J, Jammu and Kashmir, India

Sabhyata Sharma
Department of Parasitology, MAFSU, Nagpur, Maharashtra, India

Corresponding Author:
Aarushi Singh
School of Biotechnology, SKUAST-J, Jammu and Kashmir, India

Analyzing the drug release through dissolution technique of Rifampicin, Isoniazid and Pyrazinamide (3-FDC) using HPLC

Aarushi Singh, Vishaw Vikas, Narinder Panotra and Sabhyata Sharma

Abstract
A simple and efficient HPLC method for estimation of % drug release of isoniazid, pyrazinamide, and rifampicin in triple drug combination product was developed and validated for specificity, accuracy, linearity, precision and robustness ensuring suitability of the method for quantitative analysis. The % drug delivery was higher than more than 95% for all batches of isoniazid, pyrazinamide, and rifampicin. The High Performance Liquid Chromatography (HPLC) method was validated and showed to be specific, linear, precise and accurate. The dissolution test associated with HPLC developed was adequate for this purpose and could be employed for routine quality control of pharmaceutical tablets. Hence, the results indicated that this method is suitable for estimation of % Release of isoniazid, pyrazinamide, and rifampicin in a pharmaceutical formulation.

Keywords: HPLC, isoniazid, pyrazinamide, rifampicin, 3-FDC drug, dissolution testing (DT)

Introduction
High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. High performance liquid chromatography (HPLC) method plays an important role in dissolution testing (DT) procedures. It provides a wide dynamic linear range, selectivity via separation and superior sensitivity. Drug delivery refers to approaches, formulations, technologies, and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effect. It may involve scientific site-targeting within the body, or it might involve facilitating systemic pharmacokinetics; in any case, it is typically concerned with both quantity and duration of drug presence. Drug release testing, a measure of release of the active pharmaceutical ingredient (API) from the drug product matrix in controlled laboratory environment, is a key evaluation in drug development and quality control. It involves subjecting the dosage form to a set of conditions that will induce drug release and quantitating the amount of drug released under those conditions. In development, it is an essential test in assessing differences between prototypes, predicting the timeframe of API release, and modeling *in vivo* behavior. For most dosage forms to be efficacious, the API(s) must be absorbed into the systemic circulation so that it can be transported to its site of activity. This process contributes to the bioavailability of the drug substance and involves two steps: dissolution and absorption (or permeability). Dissolution testing is a requirement for all solid oral dosage forms and is used in all phases of development for product release and stability testing. It is an important tool in evaluating drug product performance for most dosage forms and is known as dissolution testing, *in vitro* release testing, and elution testing. *In vitro* dissolution testing of oral dosage forms measures the dissolution rate of an amount of drug substance going from the solid state into solution per unit time under standardized conditions. The goals of a dissolution test include prediction of bioavailability indication of the robustness of the dosage form and implication of variations in the manufacturing process. The choice of apparatus and method parameters such as medium composition, pH and sampling frequency vary depending on the dosage form and overall purpose of the test. Dissolution is commonly applied to tablets, capsules, suspensions, ointments, creams, suppositories, transdermal, implants, drug eluting stents, medicated gums, and has potential applicability with alternative formulations such as oral and injectable nano suspensions.
Isoniazid (INH), rifampicin (RIF), and pyrazinamide (PZA) are the mainstay drugs in the treatment of tuberculosis (TB). Isoniazid is the most powerful anti-tuberculosis agent against the rapidly dividing organism because it possesses the highest early bactericidal activity

(EBA). Although pyrazinamide has no or little EBA, it has a high-sterilizing activity. PZA is particularly effective against the *Mycobacterium tuberculosis* in the area of necrosis, where acidic media are available. *In vitro* studies show that both PZA and INH exhibit a concentration-dependent effect. Poor patient outcomes were associated with low levels of INH or PZA. Thus, individualization of INH and PZA doses based on their blood concentration could be helpful in optimizing the drug therapy for some patients with poor response. Several HPLC methods for the determination of one or more of the ant tuberculosis drugs in plasma or serum are available. Some of these methods are developed for determining INH alone or with its major metabolites; as a result, these methods cannot be applied for determination of PZA. HPLC methods for simultaneous determination of INH, PZA, and RIF are also available. In the present work, we describe a new HPLC method with UV detection for simultaneous determination of INH and PZA in plasma. The advantages of this method include the following: (1) a small volume of plasma is required; (2) sample pretreatment is simple; (3) the method is time efficient because the drying and reconstitution steps are omitted, and HPLC run time is short; and (4) simultaneous determination of INH and PZA is accomplished. This method is proven to be simple, fast, selective, accurate, and precise and was successfully applied for the determination of INH and PZA plasma concentrations in pulmonary TB patients.

Material and Methods

HPLC system: High performance liquid chromatography (HPLC) method plays an important role in dissolution testing (DT) procedures. It provides a wide dynamic linear range, selectivity via separation and superior sensitivity. These features have been used to solve a variety of analytical problems encountered during DT of complex drug delivery systems. The linear range for an HPLC method occurs typically up to many orders of magnitude. The wide dynamic range often allows us to conduct the DT of formulation doses ranging from 0.1 to 200 mg with a single HPLC method.

Dissolution apparatus: Paddle type, 100 RPM

Dissolution technique plays an important role in acquiring product sameness under scale up and post approval changes (SUPAC) related change. For solid dosage form, the characteristics of dissolution under physiological condition influence *in vitro* dissolution. Solubility, permeability of drug products and release products (immediate/ extended) are the major factors which affect the dissolution of development and quality control (QC) of synthetic as well as herbal drugs.

Chemical and Reagents

- **Sodium phosphate buffer:** Since the retention of ionizable compounds is very sensitive to the mobile phase pH, it is necessary to control the pH of the mobile phase by the addition of a buffer. A buffer maintains the pH when a small amount of acid or base is added. Many different substances have been used for buffering in HPLC. The most popular buffers for HPLC are phosphate and acetate. Phosphate and acetate are particularly useful buffers because they can be used at wavelengths below 220 nm.
- **Purified water:** It has advantages that it is of known purity and not prepared with equipment and glassware coming from the laboratory. This makes it an ideal for making oligo dilution and buffers for PCR reactions when preventing contamination is essential.
- **Phosphoric Acid:** Organic acids dissociate to form the negatively charged conjugate base at pH values larger than the pKa of the acid. For weak acids (e.g., carboxylic acids), the pKa values are typically in the 4 – 6 range. So, at pH values of three or lower, the acid should be present as a neutral molecule. At pH values of 7 and higher, the acids have a negative charge. The charge state of the molecule is very important in LC separations, which is why some form of pH adjustment is almost always required.
- **Methanol:** Methanol is a very common choice for the HPLC mobile phase organic solvent component in reversed phase HPLC even though acetonitrile is often superior in several aspects. On the other hand, methanol is often less expensive and less toxic than acetonitrile, so it has advantages as well.
- **Rifampicin:** A semisynthetic antibiotic produced from *Streptomyces mediterranei*. It has a broad antibacterial spectrum, including activity against several forms of Mycobacterium. In susceptible organisms it inhibits DNA-dependent RNA polymerase activity by forming a stable complex with the enzyme.
- **Isoniazid:** Isoniazid, also known as isonicotinylhydrazide (INH), is an antibiotic used for the treatment of tuberculosis. For active tuberculosis it is often used together with rifampicin, pyrazinamide.
- **Pyrazinamide:** Pyrazinamide is a medication used to treat tuberculosis. For active tuberculosis, it is often used with rifampicin, isoniazid, and either streptomycin or ethambutol. It is not generally recommended for the treatment of latent tuberculosis.

  Rifampicin/isoniazid/pyrazinamide, also known as rifampin/isoniazid/pyrazinamide, is a medication used to treat tuberculosis. It is a fixed dose combination of rifampicin, isoniazid, and pyrazinamide. It is used either by itself or along with other anti- tuberculosis medication.

Methodology

A) Dissolution technique

Speed: 100 RPM/ Paddle type

Medium: 900 ml, 10mM pH 6.8of Sodium Phosphate Buffer: Prepare it by dissolving 2.8 gm of anhydrous dibasic Sodium Phosphate in 20 litres of water and adjust the pH with Phosphoric Acid.

Time required: 45 Minutes

Test preparation

Step 1: In clean dissolution vessel, place 900 ml of de-aerated, 10 mM pH 6.8 sodium phosphate buffer.

Step 2: Allow it to attain temperature 37±0.5ºC

Step 3: Then place tablets in each vessel and start the instrument

Step 4: At the end of 45 minutes, withdraw 40 ml of sample solution from a zone midway between the surfaces of medium, top surface of the paddle but not less than 1 cm from the vessel wall**.**

Step 5: Filter the solution, about 40 ml through the Whatman No. 41 filter paper discarding first 15 ml of filtrate in separate mark test tube.

Step 6: Use the filtrate for Part A and B.

B) Preparation of working standard

Step 1:- Take 2 conical flasks each of 500 ml

Step2: Add 80 mg of Rifampicin, 40 mg Isoniazid and 200 mg of Pyrazinamide in conical flask 1 and label it as 1.
Step3:- Repeat Step 2 again and label it as conical flask 2.
Step 4:- Then add 20 ml Methanol in each standard.
Step5:- Add phosphate buffer in both the standards.
Step6:- Then sonicate both the standards.
Step7:- After sonication add buffer upto meniscus.
Step 8:- In different HPLC vials, add filtered standard solution

C) Chromatographic conditions
1) **Flow rate:**- 1.5 ml/min
2) **Wavelength:**- 238 nm
3) **Injection volume:**- 20 µl
4) **Run time:**- 20 mins
5) **Column saturation:**- Saturate the column with mobile phase before 1 hour

Mobile phase preparation
The reverse phase solvents are by convention installed on the HPLC channels A and B. The A solvent by convention and the B solvent by convention is the organic solvent acetonitrile. It is important to follow this convention since the terms A and B are commonly used to refer to the aqueous and organic solvents respectively. The Asolvent is generally HPLC grade water with 0.1% acid. The B solvent is generally an HPLC grade organic solvent such as acetonitrile or methanol with 0.1% acid. The acid is used to the improve the chromatographic peak shape. The acids used are phosphoric acid and acetic acid. A 0.1% v/v solution is made by adding 1ml of acid per liter of solvent. In our work we use acetonitrile as our organic solvent. We have heard that the best electrospray solvent is 30% methanol, 35 mM acetic acid.

a) Preparation of mobile phase A:
Step 1: Separately measure 960 ml of phosphate buffer and 40 ml of acetonitrile
Step 2: Mix and filter through 0.45 µ membrane filter paper.

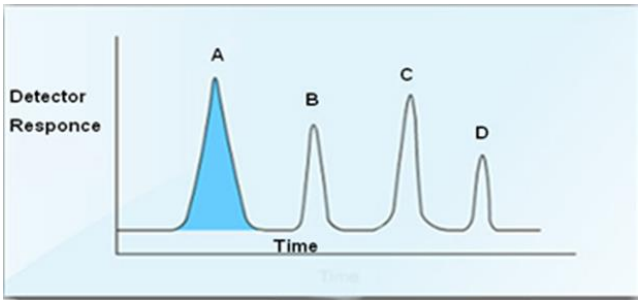
b) Preparation of mobile phase B:
Step 1: Seperately measure 450 ml of phosphate buffer and 550 ml of acetonitrile
Step 2: Mix and filter through filter paper.

Use variable mixture of solution A & B as directed in gradient program below:

Time	Concentration of A (%)	Concentration of B (%)	Elution
0	100	0	Equilibrium
0-5	100	0	Isocratic
5-6	100-0	0-100	Linear gradient
6-15	0	100	Isocratic
15-16	0-100	100-0	Linear gradient
16-20	100	0	Equilibrium

Procedure (HPLC)
Step 1:- Filter the standard and test preparation through 0.45µ membrane filter.
Step 2:- Discard first 15 ml of filtrate separately.
Step 3:- Inject 20 µl of blank.
Step 4:- Standard 1 in single injection
Step 5:- Standard 2 in five replicate injections
Step 6:- Test preparation in single injection into chromatograph
Step 7:- Then record the peak response and calculate the dissolution by using formula.
Step 8:- Now make sequence templates in the computer by running software.

Calculation of results



Calculations
Calculate the amount of Isoniazid dissolved as % as follows:-

$$\frac{AT1X\ WS1\ X\ DT\ X\ P1\ X\ 100}{AS1\ X\ DS\ X\ 100\ X\ 75}$$

Calculate the amount of Pyrazinamide dissolved as % as follows:-

$$\frac{AT2X\ WS2\ X\ DT\ X\ P2\ X\ 100}{AS2\ X\ DS\ X\ 100\ X\ 40}$$

Calculate the amount of Rifampicin dissolved as % as follows:-

$$\frac{AT3X\ WS3\ X\ DT\ X\ P3\ X\ 100}{AS3\ X\ DS\ X\ 100\ X\ 150}$$

Here:-
AT1, AT2 & AT3:- Area of test preparation for Isoniazid, Pyrazinamide & Rifampicin
AS1, AS2 & AS3:- Mean area of Standard preparation
WS1, WS2 & WS3:- Weight of standard in mgs
DS: - Dilution of test preparation
P1, P2 & P3:- Potency of standard

Results & Discussion
In drug release testing procedure, it is mandatory to have results more than 75% otherwise the drug will be considered as sub-standard or in-efficient.
The mean value of all batches of Isoniazid samples was 98.905 and standard error was 0.206 with median 99.080. The standard deviation was 0.653 with sample variance 0.426 along with skewness -0.535 and kurtosis -0.106. The range came out 2.180. (Table 4)
The mean value of all batches of Pyrazinamide samples was 99.14 and standard error was 0.14 with median 99.12. The standard deviation was 0.45 with sample variance 0.21 along with skewness 0.42 and kurtosis -0.19. The range came out 1.46. (Table 5)
The mean value of all batches of Rifampicin samples was 99.14 and standard error was 0.14 with median 99.12. The standard deviation was 0.45 with sample variance 0.21 along with skewness 0.42 and kurtosis -0.19. The range came out 1.46. (Table 6)
Drug solubility and solution stability are important properties to be considered when selecting the dissolution medium. Currently, a wide variety of chromatographic stationary phases, providing significantly different retention and

selectivity, are commercially available and principally offer the opportunity to perform any separation. However, many columns present similar characteristics, which made the selection of a proper chromatographic system difficult and problem dependent. The sharpness of a chromatographic peak was an indication of the quality of the chromatographic column and its efficacy. Small particle size and long column dimensions generally increase column efficiency, which in turn increases sensitivity, analytical resolution, and speed, though other characteristics had a greater effect on separation. Based on these investigations a simple and economic method has been developed for the estimation and efficiency of 3-FDC drug present in a combined (isoniazid, pyrazinamide, and rifampicin) pharmaceutical dosage form. Previously, a simultaneous equation method was applied to assay a mixture of rifampicin and isoniazid by UV spectrophotometry (Begum *et al*., 2013). Upon achieving optimum separation, HPLC method was validated as per ICH guidelines to ensure its suitability for routine use in estimation of % drug release of 3-FDC drug in a pharmaceutical formulation. The analysis was done for 10 batches each of Isoniazid, Pyrazinamide & Rifampicin and the result in each case was satisfactory according to the mandatory guideline of percent drug release more than 75%. HPLC method developed for dissolution of Isoniazid, Pyrazinamide & Rifampicin tablets has been proved to be simple, accurate, precise and robust to changes in analytical methodology. This method can be used for estimation of % release of Isoniazid, Pyrazinamide & Rifampicin in the pharmaceutical formulation in quality control laboratory of any pharmaceutical industry and is satisfactory.

Conclusion

A simple and efficient HPLC method for estimation of % drug release of isoniazid, pyrazinamide, and rifampicin in triple drug combination product was developed and validated for specificity, accuracy, linearity, precision and robustness ensuring suitability of the method for quantitative analysis. The % drug delivery was higher than more than 95% for all batches of isoniazid, pyrazinamide, and rifampicin. The High Performance Liquid Chromatography (HPLC) method was validated and showed to be specific, linear, precise and accurate. The dissolution test associated with HPLC developed was adequate for this purpose and could be employed for routine quality control of pharmaceutical tablets. Hence, the results indicated that this method is suitable for estimation of % Release of isoniazid, pyrazinamide, and rifampicin in a pharmaceutical formulation.

Table 1: Percent drug release of isoniazid

Batch No.	% drug release
1	99.88
2	99.25
3	99.29
4	99.16
5	98.13
6	97.70
7	98.60
8	99.00
9	98.59
10	99.45

Standard for Isoniazid	
> 75 %	Efficient
<75 %	In-efficient

Table 2: Percent drug release of pyrazinamide

Batch No.	% drug release
1	100
2	99.10
3	98.67
4	98.54
5	99.45
6	99.40
7	98.98
8	99.14
9	98.66
10	99.47

Standard for Pyrazinamide	
> 75 %	Efficient
<75 %	In-efficient

Table 3: Percent drug release of rifampicin

Batch No.	% drug release
1	100
2	99.10
3	98.67
4	98.54
5	99.45
6	99.40
7	98.98
8	99.14
9	98.66
10	99.47

Standard for Rifampicin	
> 75 %	Efficient
<75 %	In-efficient

Table 4: Descriptive statistics of isoniazid

Mean	98.905
Standard Error	0.206
Median	99.080
Standard Deviation	0.653
Sample Variance	0.426
Kurtosis	-0.106
Skewness	-0.535
Range	2.180
Minimum	97.700
Maximum	99.880
Sum	989.050
Count	10

Table 5: Descriptive statistics of pyrazinamide

Mean	99.14
Standard Error	0.14
Median	99.12
Standard Deviation	0.45
Sample Variance	0.21
Kurtosis	-0.19
Skewness	0.42
Range	1.46
Minimum	98.54
Maximum	100.00
Sum	991.41
Count	10

Table 6: Descriptive statistics of rifampicin

Mean	99.14
Standard Error	0.14
Median	99.12
Standard Deviation	0.45
Sample Variance	0.21
Kurtosis	-0.19
Skewness	0.42
Range	1.46
Minimum	98.54
Maximum	100.00
Sum	991.41
Count	10

Table 7: Coefficient of correlation between isoniazid, pyrazinamide & rifampicin

		Isoniazid	Pyrazinamide	Rifampicin
Isoniazid	Pearson Correlation	1	.119	.119
	Sig. (2-tailed)		.743	.743
	N	10	10	10
Pyrazinamide	Pearson Correlation	.119	1	1.000**
	Sig. (2-tailed)	.743		.000
	N	10	10	10
Rifampicin	Pearson Correlation	.119	1.000**	1
	Sig. (2-tailed)	.743	.000	
	N	10	10	10

**. Correlation is significant at the 0.01 level (2-tailed).

Result: - Non-Significant correlation

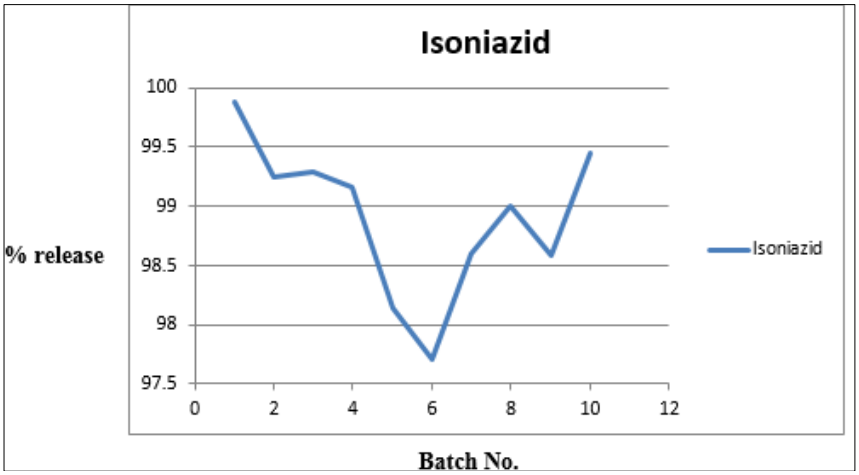


Fig 1: Graphical representation of samples vs release of isoniazid drug (%)

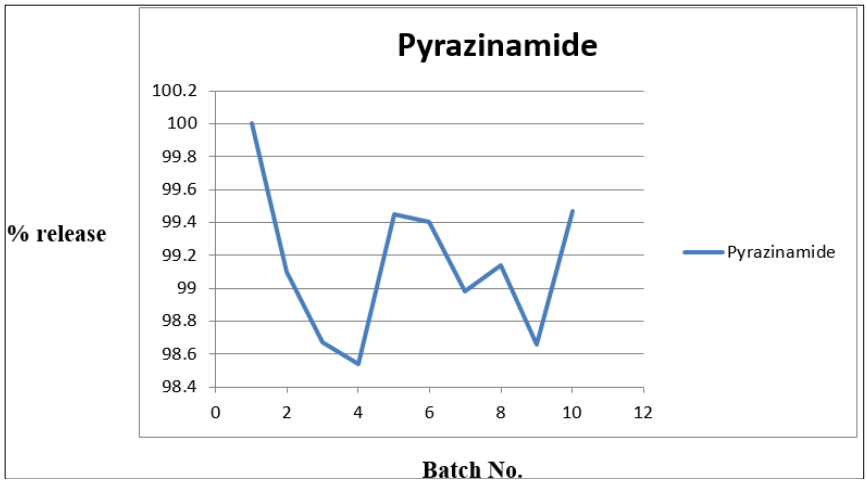


Fig 2: Graphical representation of samples vs release of pyrazinamide drug (%).

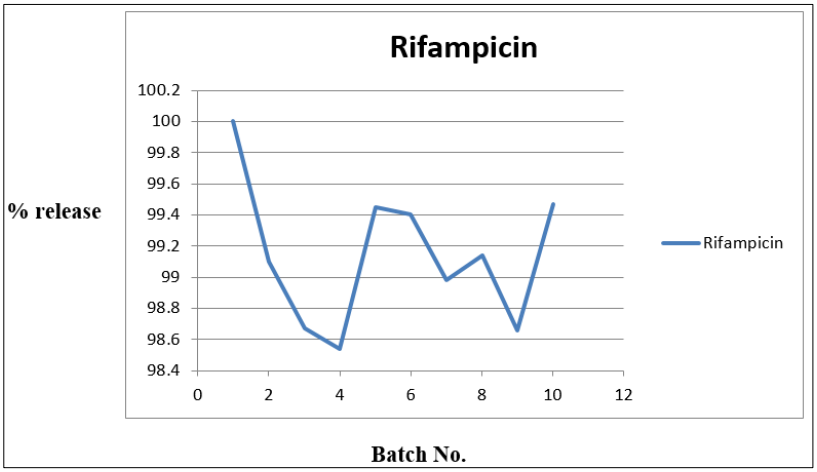


Fig 3: Graphical representation of samples vs release of rifampicin drug (%).

References

1. Dressman J, Kramer J. Pharmaceutical dissolution Testing, Taylor & Francis Group, LLC, 2005.
2. British Pharmacopoeia, HMSO Publication: London; 2009; I-II:5379.
3. Adams AIH, Bergold AM. Journal of Pharmaceutical and Biomedical Analysis. 2001; 26:505-508.
4. Chen D, Jiang S, Chen Y, Hu Y. Journal of Pharmaceutical and Biomedical Analysis. 2004; 34(1):239-245.
5. Jain DS, Sanyal M, Subbaiah G, Pande UC, Shrivastav P. Journal of Chromatography B. 2005; 829(1-2):69-74.
6. Kim KM, Jung BH, Woo JS, Paeng KJ, Chung BC. Journal of Chromatography B: Analytical Technologies in the biomedical and life sciences. 2002; 769(2):333-339.
7. Lories I, Bebawy LI, El-Kousy N, Suddik JK, Shokry M. Journal of Pharmaceutical and Biomedical Analysis. 1999; 21:133-142.
8. Lucangioli SE, Hermida LG, Tripodi VP, Rodriguez VG, Lopez EE, Rouge PD *et al*. Journal of Chromatography A. 2000; 871:207-215.
9. Mandrioli R, Saracino MA, Ferrari S, Berardi D, Kenndler E, Raggi MA. Journal of Chromatography B. 2006; 836(1-2):116-119.
10. Wiener HL. Journal of Chromatography. 1990; 527:467-472.
11. Zhou MX, Foley JP. Journal of Chromatography A. 2004; 1053:13-23.
12. Stalevo (Carbidopa, levodopa and entacapone) tablets, prescribing information, Novartis pharmaceuticals limited.
13. Ashish S, Doshi Kalpesh J, Upadhyay Tushar NM, Nanda N. Development and application of a high-performance liquid chromatographic method for the determination of *in vitro* drug release of levodopa, Carbidopa, and Entacapone from a tablet formulation. Journal of AOAC International. 2009; 92(2):394-403.
14. Clésio Paim S, Magda Martins T, Marcelo Malesuik D, Martin Steppe LC. Determination of entacapone in entacapone tablets; *in-vitro* dissolution studies. Journal of Chromatographic Science. 2010; 48:755-759.
15. Prafullachandra T, Mhatre Vinayak S, Pai NR, Maurya C, Smrut T. Estimation of Entacapone tablets by reverse phase high performance liquid chromatographic method. Bioscience Discovery. 2011; 2(3):294-298.
16. Madrakian T, Afkhami A, Borazjani M, Bahram M. Simultaneous derivative spectrophotometric determination of levodopa and Carbidopa in pharmaceutical preparations. Bull. Korean Chem. Soc. 2009; 25(12):1764-1768.
17. Bugamelli F, Marcheselli C, Barba E, Raggi MA. Determination of l-dopa, carbidopa, 3-O-methyldopa and entacapone in human plasma by HPLC-ED. Journal of Pharmaceutical and Biomedical Analysis. 2011; 54(3):562-567.
18. Raut PP, Charde SY. Simultaneous estimation of levodopa and carbidopa by RP-HPLC using a fluorescence detector: its application to a pharmaceutical dosage form. Luminescence. 2014; 29(7):762-771.
19. Gadallah MN, Mohamed MS. Determination of drug Entacapone. Journal of the Chilean Chemical Society. 2010; 55(1):85-89.
20. Mohamed Rizk, Ali Attia K, Mona Elshahed S, Amir SF. Validated voltammetric method for the determination of antiparkinsonism drug entacapone in bulk, pharmaceutical formulation and human plasma. Journal of Electroanalytical Chemistry. 2015; 743(112-119).
21. Bhatnagar P, Vyas D, Sinha SK, Chakrabarti T. Stability Indicating HPLC Method for Simultaneous Estimation of Entacapone, Levodopa and Carbidopa in Pharmaceutical Formulation. Journal of Chromatography and Separation Techniques. 2015; 9(6):304.
22. Rama Krishna VR, Murali Krishna KB, Hari Babu B. Development and validation of liquid chromatographic method for the simultaneous estimation of Levodopa, Carbidopa and Entacapone in the combined dosage form. Journal of Pharmacy Research. 2014; 8(3):281-288.
23. Monograph for levodopa and carbidopa orally disintegrating tablets, USP 37 NF. 2014; 32:2134-2136.
24. Monograph for levodopa and carbidopa extended release tablets, USP 37 NF. 2014; 32:2129-2134.
25. Monograph for levodopa and Carbidopa tablets, British pharmacopoeia. 2014; 3:357-358.
26. Monograph for levodopa and Carbidopa tablets, Indian pharmacopoeia. 2014; 2:2081-2084.
27. Monograph for Entacapone tablets, USP 37 NF. 2014; 32:2801-2802.
28. Monograph for levodopa tablets, USP 37 NF. 2014; 32:3535-3536.
29. Harshal Pawar A, Lalitha KG. Development and Validation of a Novel RP-HPLC Method for Estimation of Losartan Potassium in Dissolution Samples of Immediate and Sustained Release Tablets, Article ID 736761, 2014, 8.