IJPSR (2019), Volume 10, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 March 2019; received in revised form, 11 June 2019; accepted, 17 July 2019; published 01 December 2019

SIMULTANEOUS ESTIMATION OF AMANTADINE HYDROCHLORIDE AND OSELTAMIVIR PHOSPHATE USING PRECOLUMN DERIVATIZATION TECHNIQUE

V. Ramya [1], Zaranappa [*1] and Y. N. Munisham Gowda [2]

Department of Pharmaceutical Chemistry [1], Government College of Pharmacy, Subbaiah Circle, Bengaluru - 560027, Karnataka, India.
Department of Drugs Control [2], Drug Testing Laboratory, Palace Road, Bengaluru - 560001, Karnataka, India.

Keywords:

Amantadine hydrochloride, Oseltamivir phosphate, Precolumn derivatization, RP-HPLC, Simultaneous estimation, ICH guidelines

Correspondence to Author:
Dr. Zaranappa

Assistant Professor,
Department of Pharmaceutical Chemistry, Government College of Pharmacy, #2 P kalingarao Road, Subbaiah Circle, Bengaluru - 560027, Karnataka, India.

E-mail: zaranappa@gmail.com

ABSTRACT: A simple, efficient HPLC method has been developed and validated for simultaneous determination of Amantadine hydrochloride and Oseltamivir phosphate using pre-column derivatization with phenyl isothiocyanate (PITC). The chromatography separation was achieved on Phenomenex C18 column of 250 × 4.6 mm 5 µm particle size, mobile phase used for chromatographic run consisted of acetonitrile and water (60: 40% v/v) with a flow rate of 2 mL/min. The analysis was performed at ambient temperature, and the eluent was monitored at 250 nm using a UV detector. The retention time of Amantadine hydrochloride and Oseltamivir phosphate was found to be 7.1 min and 3.2 min. The limit of detection (LOD) for Amantadine hydrochloride and Oseltamivir phosphate was observed to be 0.1µg/mL and 0.25 µg/mL, respectively. Limit of quantification (LOQ) was found to be 0.5 µg/mL and 0.25 µg/mL. The developed method was validated as per ICH guidelines using parameters like linearity, specificity, precision, linearity, accuracy, ruggedness, robustness, LOD, and LOQ. All the validation parameters were found to be well within the acceptance criteria. Hence, the proposed method can be used for the routine analysis of Amantadine hydrochloride and Oseltamivir phosphate in bulk and tablet dosage forms.

INTRODUCTION: Amantadine is a synthetic tricyclic amine with antiviral, antiparkinsonian, and antihyperalgesic activities. Amantadine appears to exert its antiviral effect against the influenza A virus by interfering with the function of the transmembrane domain of viral M2 protein, thereby preventing the release of infectious viral nucleic acids into host cells.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(12).5443-49

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(12).5443-49

Amantadine exerts its antiparkinsonian effects by stimulating the release of dopamine from striatal dopaminergic nerve terminals and inhibiting its presynaptic reuptake and also exerts an anticholinergic effect through inhibition of N methyl-D-aspartic acid (NMDA) receptor-mediated stimulation of acetylcholine, resulting in anti-hyperalgesia [1]. Since Amantadine lacks useful chromophores in its structure and cannot be readily quantified by either UV or fluorescence detection techniques, consequently, Amantadine has to be derivatized before analysis.

Oseltamivir phosphate is an antiviral drug, a neuraminidase inhibitor used in the treatment and prophylaxis of both influenza A and influenza B [2].

This can be used prophylactically to prevent influenza during epidemics. Oseltamivir is a drug of choice for bird flu (currently strain causing a pandemic is H5N1) as well as swine flu (H1N1). Oseltamivir carboxylate has an antiviral spectrum and potency similar to that of Zanamivir. It inhibits amantadine -resistant influenza A viruses [3]. Combination chemotherapy provided a survival advantage over the single-agent treatment of mice inoculated with neurotropic H5N1 influenza virus. This strategy might be an option for the control of pandemic influenza viruses that are sensitive to Amantadine. Combination treatment with Amantadine and Oseltamivir provided greater protection against lethal infection with Amantadine sensitive H5N1 virus than did monotherapy. Moreover, the spread of the virus to the brain was prevented by both combination regimens. The efficacy of the drug combinations against Amantadine-resistant H5N1 virus was comparable to that of Oseltamivir alone [4].

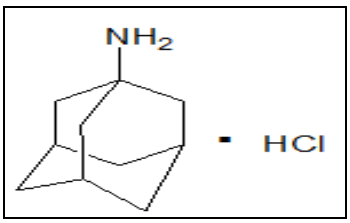

FIG 1: STRUCTURE OF AMANTADINE HYDROCHLORIDE

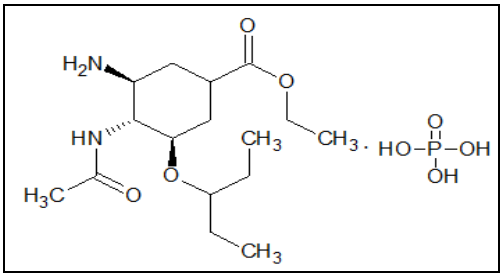

FIG 2: STRUCTURE OF OSELTAMIVIR PHOSPHATE

On literature survey that only few HPLC methods were reported on precolumn derivatization of Amantadine for individual drug [6, 7, 8, 14]. Oseltamivir phosphate was estimated and validated by RP-HPLC [9, 10, 11, 12, 13] of the individual drug. But no method was found for simultaneous estimation of Amantadine hydrochloride and Oseltamivir phosphate by RP-HPLC. Hence, there is a need for the development of a newer, rapid, accurate, and reproducible analytical method for simultaneous estimation of Amantadine hydrochloride and Oseltamivir phosphate in bulk and pharmaceutical dosage forms.

MATERIALS AND METHODS:

Chemicals and Solvents: Pure standards (API) of Amantadine hydrochloride and Oseltamivir phosphate were procured from Apotex pharmaceuticals. Phenyl isothiocyanate (PITC 99%), sodium bicarbonate and sodium carbonate (Analytical grade) were purchased from Hi-media. Acetonitrile (HPLC grade) was purchased from E. Merck. Amantadine 100 mg and Oseltamivir 75 mg capsules were procured from local market Bengaluru, India.

Instrument and Chromatographic Conditions: The High-Performance Liquid Chromatography consisted of Shimadzu-SPD-20A prominence auto-sampler fitted with UV visible detector (SPD-20A), PDA detector (SPD-M20A) with Shimadzu-LC-20AT pump. The chromatographic separation was achieved by using Phenomenex C18 (250 mm × 4.6 mm, 5µ) stationary phase and mobile phase consists of acetonitrile and water (60:40 v/v with a flow rate of 2 mL/min. The chromatogram was recorded using LC solutions software. The analysis was performed at ambient temperature, and the eluent was monitored at 250 nm using a UV detector.

Preparation of Stock and Standard Solutions: Accurately 25 mg of Amantadine hydrochloride & 25 mg of Oseltamivir phosphate were weighed into a clean and dry 25 ml volumetric flask dissolved with sufficient volume of methanol. The final volume was made up to 25 ml with methanol to give the solution containing 1 µg/mL of Amantadine hydrochloride and 1 µg/mL of Oseltamivir phosphate.

Preparation of Phenyl Isothiocyanate Solution: 1 ml of phenyl isothiocyanate was transferred into 25 ml clean and dry volumetric flask. Sufficient volume of acetonitrile was added and vortexed for 1 min, and volume was made up to the mark with acetonitrile.

Preparation of Sodium Bicarbonate Solution: 1.25g of sodium bicarbonate was weighed and transferred into a clean and dry 25 ml volumetric flask. Sufficient volume of HPLC grade water was added, vortexed for 1 min. The volume was made up to the mark with HPLC grade water.

Preparation of Sodium Carbonate Solution: 0.25g of sodium carbonate was weighed and transferred into clean and dry 25 ml volumetric flask. Sufficient volume of HPLC grade water added, vortexed for 1 min. The volume was made up to the mark with HPLC grade water.

Derivatization Procedure for Standard and Sample Solutions: Derivatization of Amantadine hydrochloride and Oseltamivir phosphate with phenyl isothiocyanate was carried out. Transfer 1 ml of Amantadine hydrochloride and Oseltamivir phosphate, 0.5 ml of phenyl isothiocyanate, 0.5 ml of sodium bicarbonate solutions were added and heated at 40 °C water bath for 10 min. After 0.5 ml of sodium carbonate was added, cyclomixed and heated 40 °C again for 5 min and the solution was cooled down to room temperature, and final volume is made up to 25 ml with water and methanol (70:30% v/v) as a diluent and filtered with 0.45 µm nylon syringe filter. 20 µl of the derivatized solution was injected, and detection was carried out at 250 nm. All the solutions were stored at 4 °C until the analysis.

Method Validation: The proposed method was validated in compliance with ICH guidelines for linearity, accuracy, precision, specificity, robustness, and system suitability parameters by the following procedures.

Linearity: Accurately 50 mg of Amantadine hydrochloride and 50 mg of Oseltamivir phosphate was weighed into a clean and dry 10 ml volumetric flasks separately, dissolved with sufficient volume of diluent. The volume was made up to 10 ml with diluent to get the concentration of 5000 µg/mL for Amantadine hydrochloride and 5000 µg/mL of Oseltamivir phosphate. From the above stock solutions, 2.5 ml of Amantadine hydrochloride and Oseltamivir phosphate were transferred into 25 ml volumetric flask and derivatized with PITC. The volume was made up to 25 ml with diluent to get the concentration of 500 µg/mL for Amantadine hydrochloride and 500 µg/mL of Oseltamivir phosphate.

Preparation of Working Standard Solutions: The various concentration of working derivatized solution of Amantadine hydrochloride and Oseltamivir phosphate was made by pipetting 0.1 ml, 0.2 ml, 0.5 ml, 1 ml, 1.2 ml and 1.5 ml from stock (II) into a series of 10 ml volumetric flask and diluted to 10 mL to get the final concentration of 5 µg/mL, 10 µg/mL, 25 µg/mL, 50 µg/mL, 75 µg/mL of Amantadine hydrochloride and 5 µg/mL, 10 µg/mL, 25 µg/mL, 50 µg/mL and 75 µg/mL of Oseltamivir phosphate solutions respectively.

Determination: The derivatized solutions of Amantadine hydrochloride from 5 µg/mL to 75 µg/mL and Oseltamivir phosphate from 5 µg/mL to 75 µg/mL were injected into a chromatograph at a flow rate of 2 ml/min.

Retention time and peak area obtained were recorded, and a standard calibration curve was plotted for Amantadine hydrochloride, and Oseltamivir phosphate and linearity equation was derived. The correlation coefficient, % curve fitting, were also calculated. The results obtained were shown in **Table 1** & **2**, **Fig. 3**.

TABLE 1: LINEARITY DATA OF AMANTADINE HYDROCHLORIDE AND OSELTAMIVIR PHOSPHATE

Amantadine hydrochloride		Oseltamivir phosphate	
Concentration (µg/mL)	**Average Area**	**Concentration (µg/mL)**	**Average Area**
5	72579	5	294569
10	142823	10	517979
25	387518	25	1258567
50	859669	50	2369122
60	1034329	60	2983276
75	1321623	75	3627917

TABLE 2: LINEARITY REPORT OF AMANTADINE HYDROCHLORIDE AND OSELTAMIVIR PHOSPHATE

Parameters	Amantadine hydrochloride	Oseltamivir phosphate	Acceptance criteria
Linearity Range	5µg/mL-75µg/mL	5µg/mL-75µg/mL	-
Regression Equation	**y** = 18071×-36475	**y** = 47814×+47156	-
Correlation Coefficient	0.9991	0.999	More than 0.999
Intercept	36475	47156	-
Slope	18071	47814	-

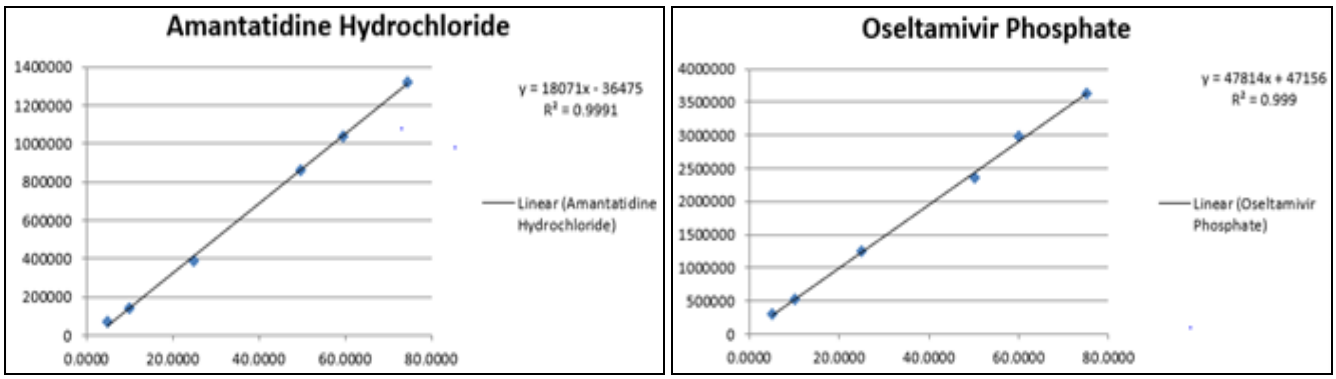

FIG. 3: STANDARD CALIBRATION CURVES

Accuracy:

Preparation of Sample Stock Solution: Twenty tablets, each containing 100 mg of Amantadine hydrochloride and 75 mg of Oseltamivir phosphate were weighed and finely powdered. Powder equivalent to 100 mg of Amantadine hydrochloride and 100 mg of Oseltamivir phosphate was taken and transferred into a clean, dry 100 ml volumetric flask. The powder was first dissolved in diluent and sonicated for 20 min. The resulting mixture was then filtered through Whatman filter no 0.45 µ. The final volume of the filtrate was made up to 100 ml with diluent

Preparation of Standard Stock Solution: Accurately weighed 10 mg of standard drug Amantadine hydrochloride and 10 mg of Oseltamivir phosphate was transferred into a clean, dry 10 ml volumetric flask and the volume was made up to 10 ml with diluent to get the concentration of 100 µg/mL of Amantadine hydrochloride and 100 µg/mL of Oseltamivir phosphate.

Preparation of Standard and Sample Mixture:

Level I (80%): Volume of 0.8 ml sample stock solution, 1.0 ml of standard solution was transferred to 10 ml volumetric flask and derivatized with PITC separately, and volume was made up to mark with diluent (three replicates).

Level II (100%): Volume of 1.0 ml sample stock solution, 1.0 ml working standard stock solution was transferred to 10 ml volumetric flask and derivatized with PITC. Volume was made up to mark with diluent (three replicates).

Level III (120%): Volume of 1.2 ml sample stock solution, 1.0 ml of working standard stock solution was transferred to 10 ml volumetric flask and derivatized with PITC. Volume was made up to mark with diluent (three replicates).

Determination: The resulting mixture was injected repeatedly into the chromatograph, the peak area and chromatogram obtained were recorded, and the % recovery of standard Amantadine hydrochloride and Oseltamivir phosphate was calculated. The results obtained are presented in **Table 3**, **4** & **5**.

Specificity: The specificity of the method was established by injecting the solutions of a placebo, standard individually to examine any interference. These results show that the peak of analyte was pure and excipients in the formulation did not interfere with the analysis.

TABLE 3: RECOVERY STUDY DATA FOR AMANTADINE HYDROCHLORIDE

Level	Replicate	Std Conc. (µg/mL)	Sample Conc. (µg/mL)	Peak area	The actual amount added (µg/mL)	Amt of std. recovered (µg/m)	% Recovery
	I	50	40	796679	802.31	848.3	105.73
80%	II	50	40	794935	802.1	846.5	105.50
	III	50	40	786555	803.0	837.5	104.39
	I	50	50	987038	1002.89	1051.0	104.80
100%	II	50	50	981868	1002.18	1045.5	104.25
	III	50	50	987387	1002.51	1051.4	104.83
	I	50	60	1167278	1203.47	1242.9	103.28
120%	II	50	60	1139263	1203.41	1213.1	100.80
	III	50	60	1167902	1203.55	1243.6	103.33

TABLE 4: RECOVERY STUDY DATA FOR OSELTAMIVIR PHOSPHATE

Level	Replicate	Std Conc. (µg/mL)	Sample Conc. (µg/mL)	Peak area	Actual amount added (µg/mL)	Amt of std. recovered (µg/m)	% Recovery
80%	I	50	40	1791392	796.80	776.8	97.49
	II	50	40	1786426	795.0	774.6	97.22
	III	50	40	1760708	796.4	763.5	95.82
100%	I	50	50	2219273	996.00	962.3	96.62
	II	50	50	2210122	996.10	958.3	96.22
	III	50	50	2202575	996.90	955.1	95.89
120%	I	50	60	2723201	1195.19	1180.8	98.80
	II	50	60	2719433	1196.0	1179.2	98.66
	III	50	60	2692810	1195.21	1167.6	97.69

TABLE 5: REPORT OF RECOVERY STUDIES FOR AMANTADINE HYDROCHLORIDE AND OSELTAMIVIR PHOSPHATE

Level	Mean % Recovery of Amantadine hydrochloride	Mean % Recovery of Oseltamivir phosphate	Acceptance criteria
80%	105.21	97.84	90-110%
100%	104.63	96.24	90-110%
120%	102.47	98.38	90-110%

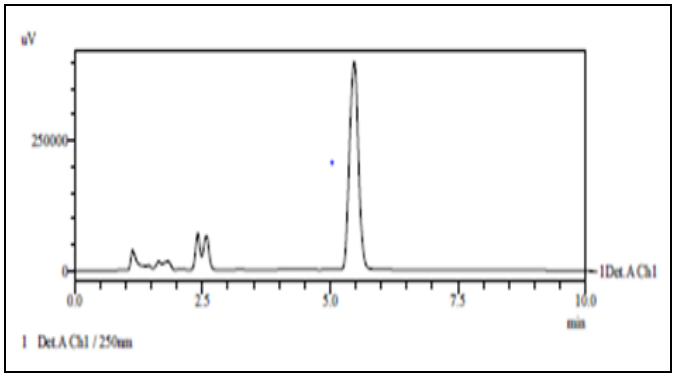

FIG. 4: PLACEBO CHROMATOGRAM OF AMANTADINE HYDROCHLORIDE AND OSELTAMIVIR PHOSPHATE

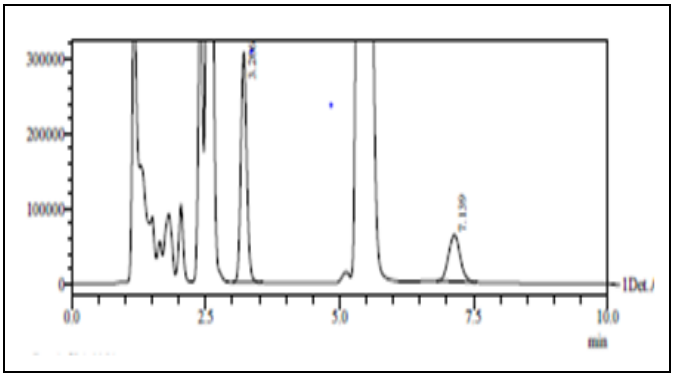

FIG. 5: STANDARD CHROMATOGRAM OF AMANTADINE HYDROCHLORIDE AND OSELTAMIVIR PHOSPHATE

The peak purity indices for sample and standard were found to be greater than 0.999, and this confirms the specificity of the method. There was no peak detected at a retention time of Amantadine hydrochloride 7.01 min and Oseltamivir phosphate 3.02 min. so, the proposed method is specific.

LOD and LOQ: LOD and LOQ for Amantadine hydrochloride and Oseltamivir phosphate by this method were evaluated based on signal-to-noise ratio method described in ICH guidelines. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit. A typical signal-to-noise ratio required for LOQ is 10:1. Using the proposed HPLC method, the LOD and LOQ values were calculated and are given in **Table 6**.

TABLE 6: DATA FOR LOD AND LOQ OF AMANTADINE HYDROCHLORIDE AND OSELTAMIVIR PHOSPHATE

Parameter	Amantadine hydrochloride		Oseltamivir phosphate	
	Peak Area	Concentration in µg/mL	Peak Area	Concentration in µg/mL
LOD	35831	0.1	12207	0.25
LOQ	50085	0.25	27528	0.5

Robustness: To evaluate the robustness of the developed RP-HPLC method, small, deliberate variations in the optimized parameters were made in chromatographic conditions like of flow rate, mobile phase ratio, and wavelength. The effect of change in flow rate, mobile phase ratio, and wavelength of detection on retention time and tailing factor were examined. The values obtained are mentioned in **Table 7**, **8** & **9**. The method was found to be unaffected by the small changes like ± 0.2 mL/min in flow-rate of mobile phase and change in mobile phase ratio from 54:46 and 66:34 and ± 2 nm in detection wavelength.

System Suitability: Six replicates of Amantadine hydrochloride and Oseltamivir phosphate sample containing were given to evaluate equipment, electronics, analytical operations, and samples suitability. Parameters calculated for system suitability were % RSD of retention time and area, number of theoretical plates and resolution. The results are given in **Table 10**.

TABLE 7: ROBUSTNESS DATA OF AMANTADINE HYDROCHLORIDE AND OSELTAMIVIR PHOSPHATE WITH CHANGE IN FLOW RATE

Change in flow rate mL/min	Peak area* of Amantadine hydrochloride	% Assay	Peak area* of Oseltamivir phosphate	% Assay
1.8	1108063	97.58	2511435	96.80
2.2	808730	98.28	2013858	97.29

TABLE 8: ROBUSTNESS DATA OF AMANTADINE HYDROCHLORIDE AND OSELTAMIVIR PHOSPHATE WITH CHANGE IN MOBILE PHASE

Change in Mobile phase ratio v/v	Peak area* of Amantadine hydrochloride	% Assay	Peak area* of Oseltamivir phosphate	% Assay
54:46	715096	102.86	1378055	99.17
66:34	657146	98.73	1278094	99.11

TABLE 9: ROBUSTNESS DATA OF AMANTADINE HYDROCHLORIDE AND OSELTAMIVIR PHOSPHATE WITH CHANGE IN WAVELENGTH

Change in wavelength in nm	Peak area* of Amantadine hydrochloride	% Assay	Peak area* of Oseltamivir phosphate	% Assay
248	970194	97.60	2276344	100.43
252	970275	97.42	2235373	101.34

TABLE 10: DATA FOR SYSTEM SUITABILITY PARAMETER FOR AMANTADINE HYDROCHLORIDE AND OSELTAMIVIR PHOSPHATE

S. no.	System Suitability Parameters	Amantadine hydrochloride	Oseltamivir phosphate	Acceptance criteria
1	Resolution	12.859	0.00	>2
2	Tailing Factor	1.09	1.139	<2
3	Theoretical plates	5034.816	3756.218	>2000

Ruggedness: Intermediate precision expresses the variations within laboratories variations: (different days, different analysts, different equipment, *etc*.). The intermediate precision was performed for Amantadine hydrochloride and Oseltamivir phosphate by a different analyst on the different instrument using a different lot of column on a different day. The % RSD for the same was calculated for Intermediate precision. The results are given in **Table 11** and **12**.

RESULTS AND DISCUSSION: Amantadine hydrochloride and Oseltamivir phosphate were derivatized with PITC successfully. The obtained derivatized solutions were analyzed.

TABLE 11: INTERMEDIATE PRECISION DATA OF ANALYST 1

	Amantadine Hydrochloride		Oseltamivir Phosphate	
Replicates	Peak Area*	% Assay	Peak Area*	% Assay
1	987038	105.70	2219273	99.98
2	981868	105.15	2210122	99.57
3	987387	105.74	2202575	99.23
4	994410	106.49	2243102	101.05
5	992728	106.31	2273478	102.42
6	968594	103.73	2257442	101.70
Mean	985338	105.5	2234332	100.66

*Average of six determinations

TABLE 12: INTERMEDIATE PRECISION DATA OF ANALYST 2

	Amantadine Hydrochloride		Oseltamivir Phosphate	
Replicates	Peak Area*	% Assay	Peak Area*	% Assay
1	705805	100.07	2203756	99.37
2	698967	99.10	2202043	99.29

3	706997	100.24	2197981	99.10
4	692805	98.23	2191970	98.83
5	692118	98.13	2190516	98.77
6	691449	98.04	2189799	98.74
Mean	698024	99.00	2196011	99.02

*Average of six determinations

Chromatographic conditions were screened for mobile phase composition, wavelength proportion and flow rate, mobile phase of acetonitrile: water (60:40 v/v) was optimized to give symmetric peak with short runtime at UV detection wavelength of 250 nm, and flow rate at 2 mL/min was found to be appropriate with adequate separation between the two drugs. Chromatogram of Amantadine hydrochloride and Oseltamivir phosphate at optimized chromatographic condition was recorded, the runtime was 10 min, and the retention times of Amantadine hydrochloride and Oseltamivir phosphate were found to be 7.1 and 3.2 min respectively.

CONCLUSION: The proposed HPLC method was found to be economical, sensitive, accurate, precise, specific and robust and can be used for the routine analysis of Amantadine hydrochloride and Oseltamivir phosphate in industry and academics. The results of linearity, accuracy, specificity, ruggedness, and robustness, proved to be within limits. The method provides selective quantification of Amantadine hydrochloride and Oseltamivir phosphate without interference from placebo.

ACKNOWLEDGEMENT: The authors are thankful to principal K. P. Channabasavaraj Government College of Pharmacy, Bengaluru for their guidance, support. We are thankful to drug testing laboratory Bengaluru for providing laboratory facilities.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

REFERENCES:

1. Amantadine hydrochloride [live search type of on internet] [cited Thu, Mar-1,2018, 02:05:55+5:30] available from: https://pubchem.ncbi.nlm.nih.gov/compound/Amantadine_hydrochloride
2. Oseltamivir phosphate [live search type of on internet] [cited Thu, Mar-1,2018, 02:05:55+5:30] available from: https://www.drugbank.ca/drugs/DB00198
3. Gobind and Sparsh: Review of Pharmacology. The Health Sciences Publisher, Edition 10th, 2016: 550.
4. Ilyushina NA, Hoffmann E, Salomon R, Webster RG and Govorkova EA: Amantadine-oseltamivir combination therapy for H5N1 influenza virus infection in mice. Antiviral Therapy 2007; 12(3): 363.
5. Bano T, Dudhe R and Kumar N: Development and validation of RP-HPLC Method for the determination of Oseltamivir phosphate API. JPRC 2015; 2(1): 69-73.
6. Wang F, Zhang S, Sheng C, Zhao XE and You J: Sensitive determination of Amantadine in microdialysis samples from rat plasma by HPLC with fluorescence detection. J Liq Chromatogr Relat Technol 2015; 38(17): 1622-8.
7. Yanamadala G and Praveen Srikumar P: A pre-column derivatization technique for the development and validation of a stability-indicating HPLC-UV method for the determination of memantine in bulk and formulations by using (2-napthoxy) acetyl chloride. Der Pharma Chemica 2014; 6(4): 169-80.
8. Vemuri SK, Krishna NR, Mohammad MJ and Nalluri BN: Analysis of Amantadine hydrochloride - phenyl isothiocyanate complex in bulk and pharmaceutical dosage forms by RP HPLC-PDA method. BJPR 2014; 4(2): 278-288.
9. Al-Bagary RI, El-Zaher AA, Morsy FA and Fouad MM: Kinetic study of the alkaline degradation of Oseltamivir phosphate and Valacyclovir hydrochloride using validated stability-indicating HPLC. Anal Chem Insights 2014; 9: 41-9.
10. Ameti A, Slavkovska J, Starkoska K and Arsova-Sarafinovska Z: A simple isocratic RP-HPLC method for quality control of Oseltamivir capsules. Macedonian Journal of Chemistry and Chemical Engineering 2012; 31(2): 205-15.
11. Malipatil SM, Jahan K and Patil SK: Development & validation of RP-HPLC method for the determination of Oseltamivir phosphate in bulk drug & in dosage. Indo-Global Journal of Pharmaceutical Sciences 2011; 1(1): 57-60.
12. Aydogmuş Z, Çaglar S and Toker S: RP-HPLC method for determination of Oseltamivir phosphate in capsules and spiked plasma. Analytical Letters 2010; 43(14): 2200-9.
13. Green MD, Nettey H and Wirtz RA: Determination of oseltamivir quality by colorimetric and liquid chromatographic methods. Emerging Infectious Diseases 2008; 14(4): 552.
14. Shuangjin C, Fang F, Han L and Ming M: New method for High-Performance Liquid Chromatographic determination of Amantadine and its analogs in rat plasma. Journal of Pharmaceutical and Biomedical Analysis 2007; 44(5): 1100-5.

How to cite this article:
Ramya V, Zaranappa and Gowda YNM: Simultaneous estimation of Amantadine hydrochloride and Oseltamivir phosphate using precolumn derivatization technique. Int J Pharm Sci & Res 2019; 10(12): 5443-49. doi: 10.13040/IJPSR.0975-8232.10(12).5443-49.