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Research Article

DRUG FORMULATIONS

An Antiviral Drug—Peramivir: Degradation and Identification of Impurities and the Endorsement of an HPLC–MS Method

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Abstract

Background: Peramivir is a neuraminidase inhibitor that serves as a transition state analogue for influenza neuraminidase, inhibiting the formation of new viruses in infected cells, and has been approved for intravenous administration.
Objective: To validate an HPLC method used to identify the degraded products of the antiviral drug peramivir.
Methods: Herein, we report the identification of compounds formed after the degradation of peramivir through acid, alkali, peroxide, thermal, and photolytic degradation. At the level of toxicology, a technique was devised for the isolation and measurement of peramivir.
Results: A sensitive and reliable LC–tandem mass spectrometry technique for the quantitative measurement of Peramivir and its impurities was developed and verified in order to comply with the recommendations made by the International Council for Harmonisation (ICH). The proposed protocol was in the 50–750 μg/mL range. Relative Standard Deviation values of less than 2.0% indicated good recovery in the range of 98.36–102.57%. Within the studied range, the calibration curves demonstrated good linearity and, in addition, the fitting of correlation coefficient was more than 0.999 for every impurity. Quantitative analysis of contaminants revealed the high efficiency at a low level.
Conclusion: Given its ability to separate degradation products, quantitative analysis is used to detect and quantify known and unknown impurities and degradants in the peramivir drug substance during routine analysis and stability studies. No significant degradation was found in peroxide and photolytic degradation studies.
Highlights: An HPLC method was developed and put to the test in order to analyze the behavior of the impurities of peramivir as they degraded when subjected to the stress conditions suggested by the ICH. Peramivir was found to be stable under peroxide and photolysis conditions but not stable or degradable when exposed to the acid, base, and thermal stress conditions. The

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method developed was extremely precise, linear, accurate, robust, and rugged. As a result, this technology has the potential to be used in the medication production process for regular impurity analysis as well as for the stability analysis of peramivir.

BioCryst Pharmaceuticals has developed an antiviral medicine for the treatment of influenza called peramivir (Rapivab) (1, 2). Figure 1 shows its structure (3, 4). Peramivir is a neuraminidase inhibitor (5, 6) that also functions as a transition state analogue (7, 8) of influenza neuraminidase. As a result, it prevents viruses from escaping from infected cells. It may be given intravenously without any adverse effects (9). There is no effect for the primary end point of median improvement on symptom relief in subjects, who had confirmed acute uncomplicated influenza infection versus placebo in the 2008–2009 intramuscular (10) (1M) peramivir phase II seasonal influenza study. This study was conducted during the 2008–2009 influenza season. On December 22, 2014, the U.S. Food and Drug Administration (FDA) granted a case of emergency use authorization for the drug peramivir. This authorization allows the intravenous administration of the medication to hospitalized patients only in situations where other available therapeutic approaches are either ineffective or unavailable. For instance, an example of this is when oseltamivir resistance develops and a patient is incapable of taking zanamivir by inhalation due to its toxicity (11, 12).

Experimental

Materials

(a) *Peramivir (RS/PER/20458963).*—Cadila Health Care Ltd, Ahmedabad.

(b) *Impurities (purity >99% determined by HPLC methods).*—Cadila Health Care Ltd.

(c) *Acetonitrile (mobile phase A).*—Merck India Pvt.

(d) The gradient of mobile phases A and B is as follows (Table1).

(e) *Formic acid (mobile phase B) and all other HPLC grade reagents.*—Rankem Company (Gurugram, Haryana).

(f) *Ultrapure water.*—Milli-Q water purification system (Thronton, Water Purification, Bedford, MA, USA).

Instrumentation and Methods

(a) *HPLC.*—A Waters Alliance LC (model 2695) monitored with a Sciex data-handling system and a photo diode array detector (model 2998) were utilized.

Table 1. Gradient programme

Time, min	Acetonitrile, %	Buffer, %
0	20	80
10	50	50
20	70	30
30	20	80
40	20	80

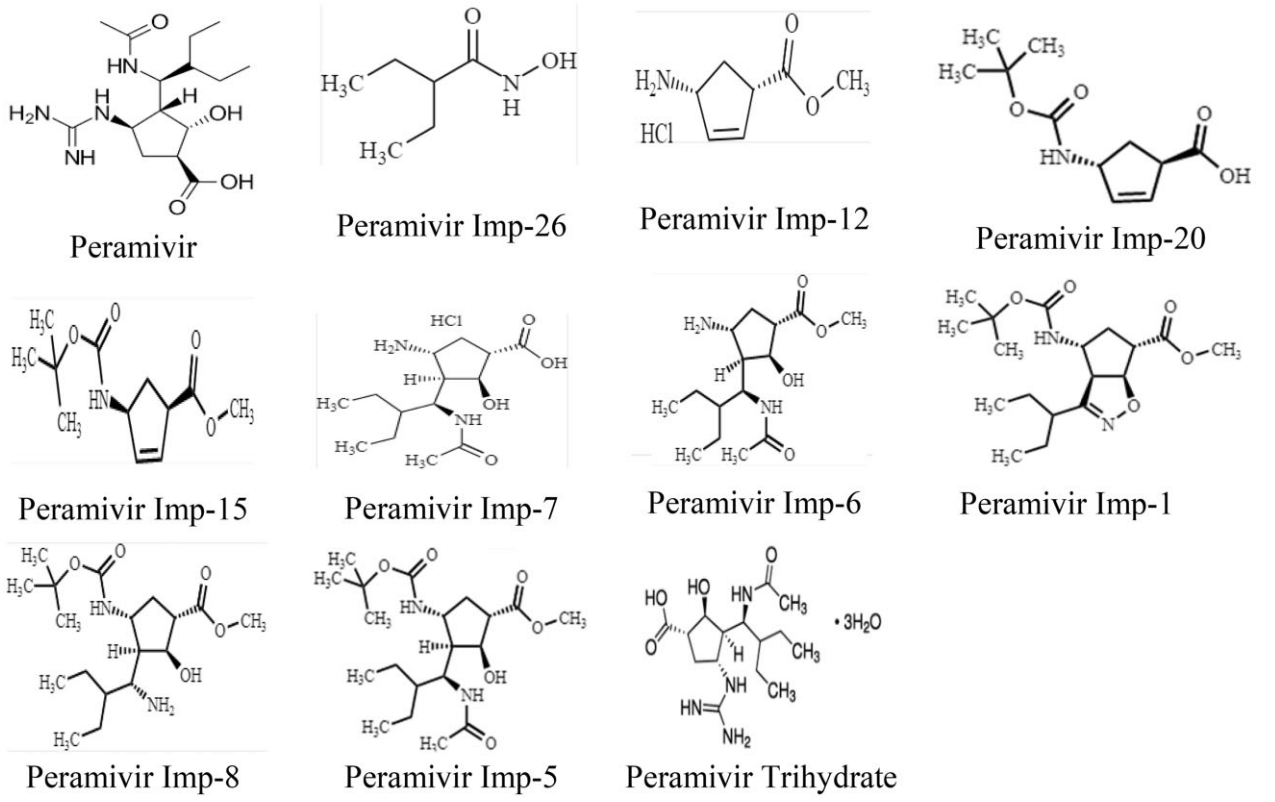

Figure 1. Chemical structures of peramivir and its related impurities.

(b) *Symmetry C18 column (150 × 4.6 mm id).*—Waters Technologies (Milford, MA, USA).

Preparation of Standard and Sample Solutions

Standard and sample solutions were prepared according to the reported literature (13). The amount of substance weighed (μg/mL) to prepare the solutions is provided in Table 2 for each compound.

(a) *Acid degradation.*—To a 10 mL volumetric flask: 1 mL sample stock solution is added, followed by 1 mL 1N HCL and left for 15 min to reach the diluent's mark 1 mL of 1N NaOH is added in the Standard Measuring Flask. The solution is filtered using a syringe filter and injected it into the HPLC system.

(b) *Base degradation.*—Into a 10 mL volumetric flask: 1 mL sample stock solution is transferred and 1 mL of 1N NaOH is added, then left for 15 min. Then to make up the volume, 1 mL of 1N HCl is added in the SMF.

(c) *Peroxide degradation.*—The solution is filtered using a syringe filter and then injected into the HPLC system. To a 10 mL volumetric flask: 1 mL sample stock solution is transferred and 1 mL 30% hydrogen peroxide solution added followed by dilution to volume with diluents. A syringe filter is used to filter the solution and injected into the HPLC system.

(d) *Thermal degradation.*—The sample solution was placed in a 105°C oven for 6 h. The resulting solution was injected into the HPLC system. The sample solution was exposed to sunlight. The solution was injected into the HPLC system (14).

Linearity

We created a calibration plot by analyzing seven solutions with concentrations ranging from 50 to 750 μg/mL. Peramivir was precisely weighed and transferred to a 100 mL volumetric flask, dissolved, and diluted to volume with mobile phase. This solution served as the stock standard solution and was gradually diluted.

LOQ and LOD

When the analyte concentration is 3.3/S, the limit of detection (LOD) value is the lowest possible value, and when the analyte concentration is 10/S, the limit of quantification (LOQ) value is the lowest possible value, both of which are used to determine a response of accuracy. To determine LOD and LOQ independently, the calibration curve approach was used. Using decreasing concentrations of standard solutions, researchers were able to predict the LOD and the LOQ for all contaminants at an SNR of 3:1 and 10:1, respectively.

Table 2. Weight used for the preparation of solutions

Compounds	Weight
Peramivir	500
Peramivir trihydrate (active metabolite)	3
Impurity-1	2
Impurity-5	2
Impurity-6	0.5
Impurity-7	1
Impurity-8	1.5
Impurity-12	0.5
Impurity-15	1
Impurity-20	1
Impurity-26	1

Accuracy and Precision

The accuracy of the relative substances method was determined by spiking peramivir test specimens in triplicate with known quantities of impurities at the levels of 50, 100, and 150%. The percentage recoveries obtained were satisfactory and are tabulated (Tables 6–8). The accuracy and precesion was examined in triplicate at three concentrations of 250, 500, and 750 μg/mL of peramivir with the different levels of 50, 100, and 150% of the targeted, and the test solution was injected three times for each spike level according to the test method. Accuracy was reported as the mean percentage recovery. For each concentration level, the RSD, %, was calculated. The precision of the proposed HPLC method was assessed by using sample mixture solutions ($n = 6$) containing 10 impurities at a concentration of 500 μg/mL.

Results and Discussion

Analytical Method Development and Validation

The ultimate aim of this research was to create a sensitive and dependable HPLC method to determine peramivir with impurities. Due to their polarities and similar structure, it is critical to separate peramivir and its ten impurities. Impurity separation at baseline level was prioritized. Consequently, as part of the preliminary work, C8 and C18 stationary phases with varying carbon loadings were used. Various mobile phases, such as acetonitrile–formic acid and methanol–formic acid solutions in various proportions, were tested. On the Symmetry C18 column (150 × 4.6 mm id; 3.5 m particle size), good peak separation was observed. Impurities in peramivir were quantified using acetonitrile–water with 0.1% formic acid as the mobile phase. The proposed method was validated according to the criteria of International Council for Harmonisation (ICH) guidelines, including specificity, linearity, LOD, LOQ, accuracy, precision, and forced degradation. Mass spectral analysis of peramivir (Figure 2) and its impurities was performed and the molecular weight confirms the availability of the title compounds.

Specificity

Specificity in analytical methods refers to the ability to assess an analyte reaction in the presence of impurities. During testing of the specificity of the suggested method for the active component, impurities were found. The chromatograms were recorded by the instrument for the injected solutions of standard, placebo, and blank. The method was specific, and when all of the peaks were present together, they were determined to be pure, and the placebo had no effect on the main peak. No peak was observed in the blank injection and Figure 3 depicts the standard chromatogram.

Linearity

At seven different concentration levels, the linearity of the method was evaluated for each impurity due to their different detection sensitivities, shown in Tables 3–5. The linear range was 50–750 μg/mL. The linear range was 50–750 μg/mL for peramivir. By using a seven-point calibration graph, linearity was satisfactorily illustrated. The slope, intercept, and regression

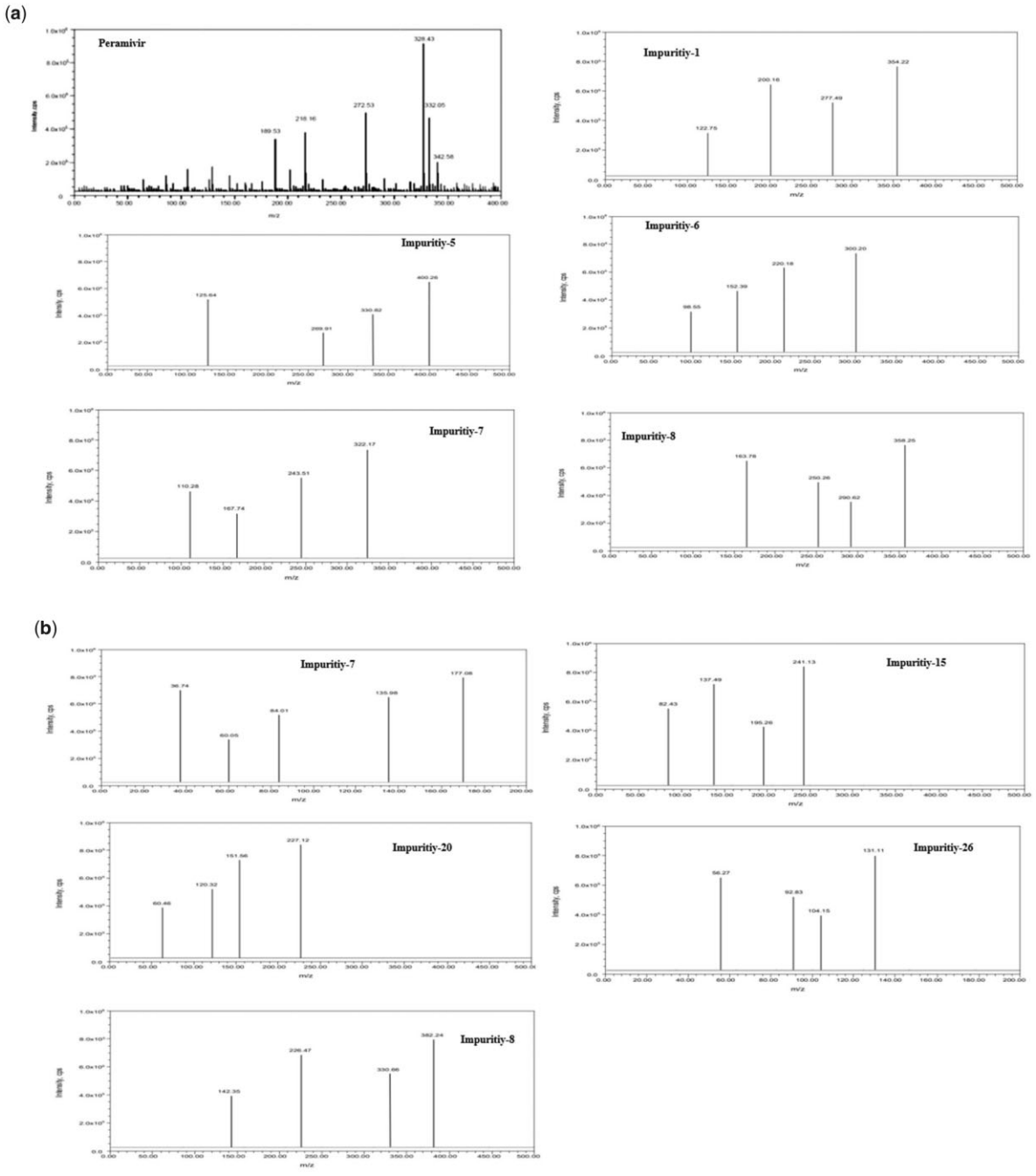

Figure 2. Mass spectra of peramivir and its related impurities.

coefficient were calculated by using least-squares linear regression analysis. All concentration levels had correlation coefficient values above 0.999.

Accuracy

The method's accuracy was tested using spiked recovery experiments. In triplicate, authentic impurities were spiked into 0.5 μg/mL peramivir at concentrations of 50% (250 μg/mL), 100% (500 μg/mL), and 150% (750 μg/mL). Tables 6 and 7 shows that good recoveries in the range of 98.48–101.52% were achieved with RSD values of less than 2.0%.

Precision

The RSD of the peak area of peramivir obtained from six duplicate injections was 0.78% when the system accuracy was analyzed. According to the analysis by precision method, the RSD for peramivir in six distinct preparations was 0.98%. The results of this method's study of peramivir have shown that it is very accurate. On several days, with different analysts, and using different HPLC equipment, the RSD values for related chemicals were less than 2%. (Table 8). This demonstrated that the method was extremely robust and stable under different parameters such as flow rate and organic phase; there was no modification in chromatographic separation and independent

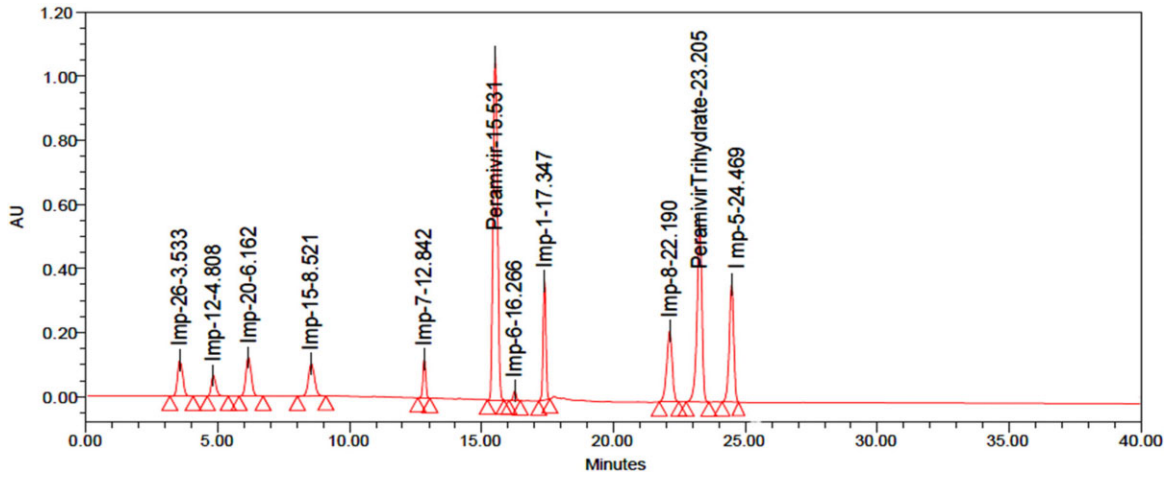

Figure 3. Chromatogram of standard.

Table 3. Linearity data from peramivir and its impurities

Linearity	Per concn	Per res	Imp26 concn	Imp26 res	Imp12 concn	Imp12 res	Imp20 concn	Imp20 res
Linearity-1	50	2 675 421	1	113 541	0.5	55 402	1	104 201
Linearity-2	125	7 206 514	2.5	250 320	1.25	135 274	2.5	278 549
Linearity-3	250	13 598 754	5	522 621	2.5	275 402	5	536 529
Linearity-4	375	20 720 561	7.5	770 218	3.75	396 528	7.5	798 652
Linearity-5	500	26 502 364	10	1 042 145	5	542 547	10	1 052 341
Linearity-6	625	33 874 157	12.5	1 258 651	6.25	668 248	12.5	1 255 204
Linearity-7	750	39 889 585	15	1 514 156	7.5	807 814	15	1 575 325
Slope	240 589.01		100 978.73		107 300.3		103 133.54	
Intercept	53 286.97		8661.22		1364.72		10 394.6	
CC	0.9997		0.9997		0.9999		0.9993	

Table 4. The linearity data of peramivir and its impurities

Linearity	Imp15 concn	Imp15 res	Imp7 concn	Imp7 res	Imp6 concn	Imp6 res	Imp1 concn	Imp 1 res
Linearity-1	1	118 496	1	99 514	0.5	53021	2	210213
Linearity-2	2.5	266 524	2.5	253 156	1.25	132054	5	532604
Linearity-3	5	566 359	5	515 692	2.5	265831	10	1024158
Linearity-4	7.5	812 546	7.5	765 214	3.75	389659	15	1542632
Linearity-5	10	1 078 421	10	103 6254	5	521478	20.00	2051478
Linearity-6	12.5	1 288 532	12.5	130 2963	6.25	662314	25.00	2568954
Linearity-7	15	154 2513	15	1 484 871	7.5	773652	30.00	3020155
Slope	102 838.03		101 309.67		103 992.25		101 269.32	
Intercept	21 444.55		4699.6		2027.05		14 297.15	
CC	0.9992		0.9993		0.9998		0.9998	

Table 5. The linearity data of peramivir and its impurities

Linearity	Imp8 conc	Imp8 res	Trihydrate conc	Trihydrate res	Imp5 conc	Imp5 res
Linearity-1	1.50	160 324	3.00	316 254	2.00	224501
Linearity-2	3.75	415 263	7.50	802 514	5.00	568945
Linearity-3	7.50	802 546	15.00	1 542 635	10.00	1163054
Linearity-4	11.25	1 236 954	22.50	2 365 924	15.00	1675482
Linearity-5	15.00	1 542 639	30.00	3 045 827	20.00	2154782
Linearity-6	18.75	1 986 574	37.50	3 865 321	25.00	2683206
Linearity-7	22.50	2 400 316	45.00	4 502 367	30.00	3245827
Slope	105 706.00		100 867.54		107 219.00	
Intercept	7713.72		31 450.18		30 420.46	
CC	0.9995		0.9996		0.9996	

Table 6. Accuracy results for peramivir and its impurities: concentration

Amount spiked, %	Peramivir	Peramivir trihydrate	Imp-1, 5	Imp-8	Imp-26, -20, -15, 7	Imp-12, 6
50	250	15	10	7.5	5	2.5
100	500	30	20	15	10	5
150	750	45	30	22.5	15	7.5

Table 7. Accuracy results of peramivir and its impurities: recovery

	Amount spiked		
Component	50	100	150
Peramivir	100.25	101.54	99.63
Impurity-26	99.03	100.46	100.05
Impurity-12	98.45	99.67	100.17
Impurity-20	99.37	99.34	100.58
Impurity-15	100.54	98.97	98.75
Impurity-7	100.23	99.26	99.36
Impurity-6	101.01	100.04	100.12
Impurity-1	100.42	100.49	100.04
Impurity-8	100.67	100.53	98.34
Peramivir trihydrate	98.86	101.75	99.27
Impurity-5	99.37	99.47	99.65

Table 8. Intermediate precision results for peramavir and its impurities

Ruggedness	Parameter	Spiked impurities	Total impurities	Purity (100 – total impurities)
Different days	Day 1	5.29	5.29	94.71
	Day 2	5.21	5.21	94.79
Different analysts	Analyst 1	5.23	5.23	94.77
	Analyst 2	5.37	5.37	94.63

Table 9. Robustness results for preamivir and its impurities

Robustness	Parameter	Spiked impurities	Total impurities	% Purity (100 – Total impurities)
Flow rate	Flow Plus	5.41	5.41	94.59
	Flow Minus	5.44	5.44	94.56
Mobile phase	Plus	5.37	5.37	94.63
	Minus	5.52	5.52	94.48

impurities, and the total impurities content remained constant under pre-planned conditions, and chromatographic separation was not affected (13–16). The findings of % purity are shown in Table 9. As a direct consequence of this precision method, the technique that was created was solid and reliable.

LOD and LOQ

LOD and LOQ of the minimum concentration (μg/mL) at which the analyte is accurately detected, quantified by the use of consistency formulae. LOD and LOQ values of peramivir and its related contaminants are given in Table 10.

Forced Degradation Studies

Acid and base hydrolysis, peroxide, reduction, photolytic, hydrolysis, and thermal degradation were all applied to the active drug (Table 11). To ensure peak homogeneity, the samples were analyzed with a photodiode array detector under these stress conditions at an initial concentration of 500 μg/mL of peramivir. The following are the results of the degradation tests. 1 N HCl at 60°C for 15 min, 1 N NaOH at 60°C for 15 min, peroxide degradation (30% H_2O_2 at 15 min), thermal degradation, and photolytic degradation No significant degradation was found in peroxide and photolytic degradation studies. Table 12 shows the results of forced degradation with the mass spectral data from the investigated compounds.

Conclusions

An HPLC protocol was devised and verified to investigate the degradation activity of peramivir and related impurities was carried out using the protocols suggested by ICH (13). Peramivir was found to be unstable against acid, alkaline, and thermal conditions, but stable under peroxide and photolysis conditions. The devised method was incredibly accurate, linear, accurate, robust, and tough. Based on the findings, this method might be

Table 10. LOD and LOQ results

	LOD		LOQ	
Component	Concentration	SNR	Concentration	SNR
Peramivir	0.625	9	2.063	28
Imp-26	0.013	5	0.001	23
Imp-12	0.006	4	0.02	22
Imp-20	0.013	5	0.043	23
Imp-15	0.013	5	0.043	23
Imp-7	0.013	5	0.043	23
Imp-6	0.006	4	0.02	22
Imp-1	0.025	6	0.083	26
Imp-8	0.019	5	0.063	24
Peramivir trihydrate	0.038	7	0.124	27
Imp-5	0.025	6	0.083	26

Table 11. Forced degradation results of peramivir

Degradation condition	Degradation of peramivir
Acid	13.6
Alkali	12.4
Peroxide	2.1
Thermal	10.9
Photolytic	1.3

Table 12. *m/z* values of peramivir impurities

Component	*m/z*
Peramivir	328.43
Peramivir trihydrate	382.24
Impuritiy-1	354.22
Impuritiy-5	400.26
Impuritiy-6	300.20
Impuritiy-7	322.17
Impuritiy-8	382.24
Impuritiy-12	177.08
Impuritiy-15	241.13
Impuritiy-20	227.12
Impuritiy-26	131.11

used as a procedure for routine impurity detection in pharmaceutical manufacturing as well as peramivir stability analysis. Future research is to investigate the toxicological and biological properties of peramivir impurities will be done in the near future.

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Conflict of Interest

None declared.

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