Innovated formulation of oseltamivir powder for suspension with stability study after reconstitution using a developed ion-pair reversed phase high-performance liquid chromatography method

Kahtan Jassim Hasson
Department of Pharmaceutics, College of Pharmacy, Al- Farahidi University, Baghdad, Iraq

Abstract
Oseltamivir is an antiviral neuraminidase inhibitor used for the treatment and prophylaxis of influenza infection with viruses A and B. The mechanism of oseltamivir antiviral activity is by inhibiting the activity of the viral neuraminidase enzyme present on the surface of the virus, which stops viral replication and infectivity. Oral suspensions of oseltamivir phosphate are dispensed orally capsules and suspension. However, the use of oral suspension for pediatric administration is preferable and is prepared as a powder for suspension. The reconstituted suspension degrades rapidly within a few days. The objective of this work is to establish a stable formulation of oseltamivir phosphate as a suspension and to assure the stability conditions for prolonged use after reconstitution in aqueous form. In addition, this required formulation should maintain a high rate of dissolution, which subsequently leads to higher bioavailability. In this study, oseltamivir forms an inclusion complex with the natural and safe polymer hydroxypropyl beta-cyclodextrin, which resembles a host because its structural cavity carries the oseltamivir molecule in the aqueous preparation and provides a protective property against environmental challengers. In addition, a high-performance liquid chromatography (HPLC) stability-indicating method of analysis has been developed using an ion-pair reversed-phase HPLC technique that is validated for precision, accuracy, reproducibility, and specificity for the determination of oseltamivir in suspension. The results of this work show the relatively long shelf life of the reconstituted oseltamivir oral powder for suspension in the new pediatric formulation, and the developed HPLC method was precisely suitable for stability study.

Key words: Degradation, hydroxypropyl beta-cyclodextrin, inclusion complex, liquid chromatography, validation

INTRODUCTION
Oseltamivir is an antiviral drug used for the treatment and prophylaxis of influenza infections with viruses A and B. The antiviral activity of oseltamivir is by blocking
the activity of the viral neuraminidase enzyme found on the surface of the virus, thereby preventing viral replication and infectivity.\(^1\) It is extensively used in medicine for adults and children as its efficacy and safety in the treatment of influenza have been clinically approved.\(^2\) Recently, a new highly potent derivative of oseltamivir has been synthesized as a selective inhibitor of influenza virus neuraminidase.\(^3\) Oseltamivir phosphate is fairly soluble in water, but it is unstable and decomposed rapidly in an aqueous solution, resulting in the formation of a carboxylate derivative. There was no problem with solid dosage forms of oseltamivir as capsules, but the problem of degradation appeared clearly in the formulation of oseltamivir oral powder for suspension after reconstitution with water which does not last for few days to be out of specifications. It is recommended that the oseltamivir powder suspension for pediatric use should be maintained with a convenient dose due to the faster rate of clearance of the active metabolite oseltamivir carboxylate in children than in adults.\(^4\)

The stability of oseltamivir phosphate in aqueous preparations has been previously studied, and some results showed that the degradation increases in alkaline medium, high temperatures of storage, and with the use of unpurified water for preparation.\(^5\) Other stability studies indicated that oseltamivir phosphate in aqueous solution under forced conditions of heat undergoes acidic, alkaline, and oxidative degradation, but the alkaline condition showed a relatively higher percentage of degradation.\(^6\) Some workers revealed that oseltamivir phosphate suspension samples without sorbitol or alcohol, which were stored at 2°C–8°C and packed in low-actinic plastic containers, were stable for 30 days.\(^7\) Several analytical methods have been applied to determine oseltamivir in biological fluid and in the reconstituted suspension, using the high-performance liquid chromatography (HPLC) technique.\(^8\)–\(^10\) In this present study, an attempt was made to develop a specific HPLC method for the determination of oseltamivir in pharmaceutical preparations, particularly in suspension dosage form, which should be validated for assay and able to detect the degradation products to follow the stability studies. On the other hand, some formulation experiments of oseltamivir phosphate powder for suspension were carried out in our laboratory to achieve good stability and a reasonable shelf life after reconstitution of the suspension. Therefore, oseltamivir was formulated as an inclusion complex with hydroxypropyl beta-cyclodextrin (HPB-CD), the new derivative of beta-cyclodextrin, where the molecule of oseltamivir could be included in the structural cavity of this cyclic oligosaccharide, leading to increased solubility and improving the stability of the included compound. Several studies have discussed the use of beta-cyclodextrin and its hydroxypropyl derivative as natural, safe, and nontoxic substances used to improve the stability and dissolution of drugs.\(^11\)–\(^13\)

**MATERIALS AND METHODS**

HPB-CD and oseltamivir phosphate were obtained from Safa Company of Pharmaceutical Production (Iraq). The instrumental analysis (infrared [IR] spectrometer) and the liquid chromatography apparatus (Bruker Co.) were carried out in the laboratory of the same company. Differential Scanning Calorimetry (DSC) test was done in the Research Center of Baghdad University. Acetonitrile and Methanol of HPLC grade, the chemicals; dihydrogen phosphate, sodium lauryl sulfate (SLS), hydrochloric acid, and sodium hydroxide, all of (BDH grade).

**Make an inclusion complex**

By mixing the weight of oseltamivir phosphate powder with (HPB-CD) in a molar ratio of 1:1, then moistening the mixture with ethanol solvent. Pass the resulting mass through a No. 80 sieve and dry the fine particles in a hot air cabinet set to 50°C. The resulting complex mixture powder was subjected to IR-spectrometry analysis and DSC thermal analysis to ensure that no changes in the physicochemical properties of the parent compound (oseltamivir phosphate) may have occurred.

**The method of analysis**

As long as the stability study needs an accurate and sensitive method of analysis that should be able to identify the degradation product, some experiments were done to develop an HPLC method for this demand. Obviously, the degradation products of oseltamivir will possess higher polarity properties than the parent compound as carboxylate derivative and oxidation product formations which mostly are characterized as polar compounds. These degradation products will show peaks at different retention times, which may interfere with the retention time of the parent compound; therefore, it is necessary to use an ion-counter substance as sodium lauryl sulfate during the chromatographic process to separate the components of the sample according to their affinity of binding with the ion-counter substance in the mobile phase. Finally, the selected mode of chromatography in this case was the ion-pair reversed-phase chromatography. The established conditions for chromatography were specified as follows: using column C18, 25 cm in length, the mobile phase consists of acetonitrile 60:40 phosphate buffer 0.05M with 0.05% SLS, the pH was adjusted to 4.0, the flow rate of the mobile phase was 1 ml/min, and the detection of the components by ultraviolet at 220 nm. The performance of this chromatographic method was validated for precision, accuracy, reproducibility, and specificity in determination of oseltamivir phosphate with the sensitivity of the detection

**Formulation and stability study**

Four different formulations of Oseltamivir phosphate powder for suspension were prepared and subjected to storage effects to evaluate the proper compounding. The components of these four formulations (F1, F2, F3, and F4) are
listed in Table 1, which is reconstituted with purified water up to 100 ml. All the four formulations contain oseltamivir phosphate equivalent to 6 mg of oseltamivir base per each milliliter of reconstituted suspension for pediatric use. In addition to the active substance, the formula of suspension contains the following additives: sodium benzoate 0.05% as a (preservative), saccharine sodium 0.2% (sweetening agent), monosodium citrate (buffering agent to give pH between 4.5 and 5.0), ethylenediaminetetraacetic acid (EDTA) sodium 0.01% in F3 and F4 only (chelating agent), xanthan gum 0.2% (suspending agent), sorbitol 17% (sweetening agent), and suitable flavor. Samples of these formulations were prepared according to their contents in the form of oral liquid suspension by the addition of water up to 100 ml and packed in low-actinic plastic containers, then stored at two different temperatures at 2°C–8°C and the other part of the samples were stored at 25°C and 65% RH. The stored samples of oseltamivir reconstituted suspension were tested at different periods by the developed HPLC method and the percentages of oseltamivir in the sample were calculated by making triple injections in the chromatogram, and the average of peak areas' value was recorded for each storage time.

RESULTS AND DISCUSSION

The inclusion complexation shows no change in the nature and the physicochemical properties of oseltamivir since the DSC graphs of pure oseltamivir and that of its inclusion complex with HPB-CD exhibit the same characteristic endothermic peak of oseltamivir at 205°C–206°C, [Figure 1]; however, the other thermal peaks shown in the DSC graph at 103°C indicated the formation of a complex with HPB-CD [Figure 2].

The Fourier-transform IR spectrum of the oseltamivir complex did not show any significant change in the characteristic transmittance peak of oseltamivir at 1722 cm⁻¹, Figure 3 for pure oseltamivir phosphate and Figure 4 for the oseltamivir phosphate complex where the drug molecule response is overlapped by the presence of HPB-CD.

Table 1: The ingredients of studied formulations of oseltamivir phosphate oral powder for suspension

| Ingredients                          | F1 | F2 | F3 | F4 |
|--------------------------------------|----|----|----|----|
| Oseltamivir phosphate pure           | +  | +  | −  | −  |
| Oseltamivir phosphate HPB-CD complex | −  | +  | +  | +  |
| Sodium benzoate                      | +  | +  | +  |   |
| Saccharine sodium                    | +  | +  | +  |   |
| Monosodium citrate                   | +  | +  | +  |   |
| EDTA sodium (0.01%)                  | −  | −  | +  |   |
| Xanthan gum                          | +  | +  | +  |   |
| Sorbitol                             |   | +  | +  | −  |

+: Presence, −: Absence. EDTA: Ethylenediaminetetraacetic acid, HPB-CD: Hydroxypropyl beta-cyclodextrin
of the ionic-counter SLS increased. The retention time of oseltamivir is shown to be about 20.5 ± 0.5 min for 1 mg/mL of oseltamivir solution [Figure 5].

The validation tests of the developed HPLC method:

a. Precision: Different dilutions of oseltamivir phosphate solutions were prepared within the concentrations of 1, 2, 3, 4, and 5 mg per 100 ml and assayed by this HPLC method. The resulted peak areas were as follows: 0.231, 0.510, 0.852, 1.101, and 1.410, respectively, and a straight-line relationship was obtained. [Figure 6]

This finding indicates the high precision of the applied HPLC method for the determination of oseltamivir in solution.

b. Accuracy: Different concentrations of oseltamivir solutions were prepared accurately with the range (100 mg, 80 mg, and 120 mg per ml) and subjected to HPLC test by the application of this method comparing with a known standard solution. The resulted percentages of oseltamivir as average values of triple injections in the chromatogram were 100.1, 80.05, and 120.07, respectively.

These data indicate the high degree of accuracy in the assay test of oseltamivir.

c. Reproducibility: Five applications of oseltamivir solution of a definite concentration were injected in the chromatogram of this HPLC method for determination of the peaks areas of these applications and are recorded as follows:

Peaks areas of five repeated injections of same sample of Oseltamivir by HPLC were; 1.645, 1.623, 1.653, 1.636, and 1.648 and the standard deviation of these reading was (±0.012). the calculated relative standard deviation value (RSD) in this test was 0.64. The US pharmacopeia stated that the efficient HPLC method should not show RSD value more than 2, therefore the developed HPLC method has proved its efficiency in determination of Oseltamivir.

d. Specificity: The chromatogram of oseltamivir analysis did not show any other peak rather than the principal peak; however, in the case of testing forced degraded oseltamivir by the alkali condition, there was a secondary peak appeared at about 2.5 min. This result indicates the specificity of HPLC method for the determination of oseltamivir in aqueous solution and the suitability for stability work.

Stability study

The suggested formulations of oseltamivir phosphate suspension (for reconstitution) showed the following results on storage at two different conditions [Tables 2 and 3].

The above results of oseltamivir suspension after reconstitution on storing indicated an improvement in the stability by using the inclusion complex of oseltamivir in the formulation. Formula (F3 and F4) contains other additives such as EDTA sodium to prevent the action of metal ions in water, which may accelerate oxidation. Sorbitol-free
Table 3: Oseltamivir suspension after reconstitution stored at 25°C

| Formula | Initial percentage of oseltamivir | Remained percentage of oseltamivir on 7 days | Remained percentage of oseltamivir on 20 days | Remained percentage of oseltamivir on 40 days | Remained percentage of oseltamivir on 80 days | Remained percentage of oseltamivir on 90 days |
|---------|----------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| F1      | 100.1                            | 100.0                                       | 98.5                                       | 91.3                                       | 90.4                                       | 89.3                                       |
| F2      | 100.06                           | 100.06                                      | 100.04                                     | 99.1                                       | 98.6                                       | 95.2                                       |
| F3      | 100.0                            | 100.0                                       | 100.0                                      | 99.8                                       | 99.6                                       | 99.6                                       |
| F4      | 100.1                            | 100.1                                       | 100.1                                      | 100.0                                      | 100.0                                      | 100.0                                      |

Figure 6: The straight-line relationship between the peak area and their sample concentrations with eq. 
\( y = 0.295x - 0.05 \)

formula (F4) did not show any significant effect on results, but omitting sorbitol from the formula changed the pleasant and sweet taste of the product.

CONCLUSION

The inclusion of HPB-CD in the formulation of the inclusion complex with oseltamivir phosphate improved the stability of oseltamivir in suspension after reconstitution and prolonged the shelf life of the product over 90 days, and the complexation did not affect the nature of the active substance. The addition of EDTA sodium 0.01% in the formulation of oseltamivir suspension has potential value in stabilizing the product. The developed ion-pair reversed-phase HPLC method has been shown to be precise, accurate, and reproducible, as well as capable of detecting oseltamivir suspension degradation products.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Sweetman SC, Martindale. The Complete Drug Reference. 36th ed. China: Evertest Printing Co, Ltd.; 2009; p. 900.
2. Treanor JJ, Frederick G, Peter S, Rick B, Robert B, Dennis R, et al. Efficacy and safety of the oral inhibitor oseltamivir in treating acute influenza. J Am Med Assoc 2000;283:1016-24.
3. Jia R, Zhang J, Bertagnin C, Cherukupalli S, Ai W, Ding X, et al. Discovery of highly potent and selective influenza virus neuraminidase inhibitors targeting 150-cavity. Eur J Med Chem 2021;212:113097.
4. Oo C, Barrett J, Hill G, Mann J, Dorr A, Dutkowski R, et al. Pharmacokinetics and dosage recommendations for an oseltamivir oral suspension for the treatment of influenza in children. Paediatr Drugs 2001;3:229-36.
5. Albert K, Bockshorn J. Chemical stability of oseltamivir in oral solutions. Pharmazie 2007;62:678-82.
6. Upmanyu N, Porwal P. Degradation behavior of oseltamivir phosphate under various stress conditions using stability-indicating HPLC method. J Pharm Sci Technol Manag 2019;3:1-11.
7. Voudrie Li MA, Allen DB. Stability of oseltamivir phosphate in SysRspend SF, cherry syrup, and SysRspend SF (for reconstitution). Int J Pharm Compd 2010;14:82-6.
8. Bahrami G, Mohammadi B, Kiani A. Determination of oseltamivir carboxylic acid in human serum by solid phase extraction and high performance liquid chromatography with UV detection. J Chromatogr B Analyt Technol Biomed Life Sci 2008;864:38-42.
9. Lindegårdh N, Hien TT, Farrar J, Singhasivanon P, White NJ, Day NP. A simple and rapid liquid chromatographic assay for evaluation of potentially counterfeit Tamiflu. J Pharm Biomed Anal 2006;42:430-3.
10. Narasimhan B, Abida K, Srinivas K. Stability indicating RP-HPLC method development and validation for oseltamivir API. Chem Pharm Bull (Tokyo) 2008;56:413-7.
11. Ueda H, Ou D, Endo T, Nagase H, Tomono K, Nagai T. Evaluation of a sulfobutyl ether beta-cyclodextrin as a solubilizing/stabilizing agent for several drugs. Drug Dev Ind Pharm 1998;24:663-7.
12. Croye MA, Cheng X, Wilson JM. Development of formulations that enhance physical stability of viral vectors for gene therapy. Gene Ther 2001;8:1281-90.
13. Kang J, Kumar V, Yang D, Chowdhury PR, Hohl RJ. Cyclodextrin complexation: Influence on the solubility, stability, and cytotoxicity of camptothecin, an antineoplastic agent. Eur J Pharm Sci 2002;15:163-70.