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ORIGINAL ARTICLE

A Fourier Transform Infrared Spectrophotometry Method Used For Oseltamivir Determination in Pharmaceutical Formulations

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ABSTRACT

A Fourier transform infrared (FT-IR) spectrometric method was developed for the rapid, direct measurement of oseltamivir phosphate (OP) in pharmaceutical formulations. Conventional KBr-spectra were compared for best determination of the active substance in pharmaceutical preparations. The Beer-Lambert law and two chemometric approaches, partial least squares (PLS) and principal component regression (PCR+) methods, were used in data processing.

Key words: FT-IR analysis, oseltamivir, chemometric methods, pharmaceutical analysis.

1. INTRODUCTION

Infrared spectrometry (IR) provides a useful way for the identification of drugs [1-7] as well as for quantitative analysis, and Fourier Transform (FT-IR) technique permits continuous monitoring of the spectral baseline and simultaneous analysis of different components of the same sample.

Oseltamivir phosphate (OP, ethyl-(3*R*, 4R, 5S)-5-amino-4-acetamido-3-(pentan-3-yloxy) cyclohex-1-ene-1-carboxylate) is the first orally available inhibitor of influenza virus neuraminidase, an enzyme involved in the release of new virus particles from infected cells. The structure of oseltamivir shows it possesses a hydrophobic moiety (Fig. 1) which is responsible for its

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poor oral absorption; thus, the phosphate salt has been developed that allows oral administration of this drug. **OP** is a prodrug that is rapidly and extensively metabolized via hepatic esterases to oseltamivir carboxylate, the active form.

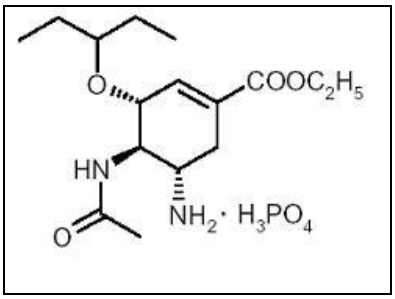

Figure 1. Oseltamivir phosphate (**OP**)

There are a few methods available for the quality control of **OP** determination in Tamiflu® capsules and generic versions. These include: HPLC, [8, 9] spectrofluorimetry [10] and capillary zone electrophoresis. [11]

Determination of the major component in drugs with FT-IR spectrometry provides an enormous amount of spectroscopic information about a sample. Chemometric methods, such as principal component regression (PCR+, Improved Principal Component Regression) and partial least-squares (PLS2, Multicomponent Partial Least Squares) analysis are commonly used to extract the specific information relevant to the analyte of interest from the full spectrum. [1, 12] These two techniques yields more accurate calibration models compared with multiple linear regressions (MLR) where a restricted set of absorption bands is used in the calibration. [13] The partial least squares (Projection to Latent Structures, PLS) regression method was developed by Wold [14] in 1966. There is a substantial amount of literature devoted to the theoretical elucidation of properties of PLS algorithm. A good introduction to the method is given by Geladi and Kowalski. [15]

Chemometric techniques which are known as numerical techniques are useful for the spectrophotometric resolution of complex mixtures of analytes without the need of prior separation or extraction. Although both PCR and PLS give succesuful results, they have several disadvantages such as using abstract mathematical theory and various softwares.

The purpose of this study is to investigate the the possibility of the application of FT-IR spectrometry in **OP** determination in pharmaceutical formulations such as *Taminil – N* capsules and to develop a fast and accurate quantification method of **OP** in these commercial formulations, using Beer's law and/or chemometric methods (PCR+, PLS1 or PLS2), thus reducing the sample pre-treatment and providing direct FTIR measurement.

2. EXPERIMENTAL

Apparatus

Data acquisition was performed using a Spectrum100 System FT-IR spectrometer equipped with Spectrum for Windows v.5.01 (Perkin Elmer Co., Beaconsfield, Bucks, UK). This software also provided a complete processing of the spectra measured. For quantitative determination special softwares were used: Spectrum Beer's law and Spectrum Quant+, respectively.

Reagents and materials

For fused KBr disk preparation a potassium bromide IR spectral grade was used (Sigma Aldrich). The standard of **OP** was supplied by F. Hoffmann - La Roche Ltd., Basel, Switzerland, while the pharmaceutical formulation Taminil-N (containg 98.55mg oseltamivir base) was manufactured by Nile Company for Pharmaceutical and Chemical Industries, Cairo, Egypt.

Recommended procedures

Taking into consideration the heterogenity of the specimens, major attention was paid to the sampling stage. Drug samples were grinded and homogenization with KBr were achieved by using a 'vibrator' ball mill (WIG-L-BUG). The temperature was kept around 25^0C and the humidity was kept at a steady level in the laboratory.

Conventional fused KBr disk spectra were recorded between 4000 and 400 cm^{-1}, by averaging 64 scans for each spectrum with a resolution of 4cm^{-1} (data point resolution / interval 1cm^{-1}) with a deuterated trigylcine sulfate DTGS detector. The samples were prepared by compressing 2.0 mg of sample with spectral grade KBr, while the background was spectral grade KBr. Each drug sample spectrum was collected three times for the same cup after rotation with 120^0. The mean of the spectra was then used in the following analytical steps.

For calibration, conventional fused KBr disk spectra were recorded with a DTGS detector from samples prepared by compressing a standard substance **OP** in spectral grade KBr (calibration was made using four points 0.25mg, 0. 5mg, 1.0mg and 1.5 mg, respectively). The calibration procedure is based on either a modified form of principal component regression (PCR) or on a partial least squares (PLS) fit for one or more properties. The regression model for each property is refined by selecting only those factors considered to be of statistical significance in determining it.

Experimental parameters, such as calibration methods, (PCR+, PLS1 or PLS2, respectively) were compared and recommendations on the best options for **OP** analysis were made.

3. RESULT AND DISCUSSION

Figure 2 presents the mean spectra for OP samples using the KBr disk method while the spectra of pharmaceutical drug is presented in Figure 3.

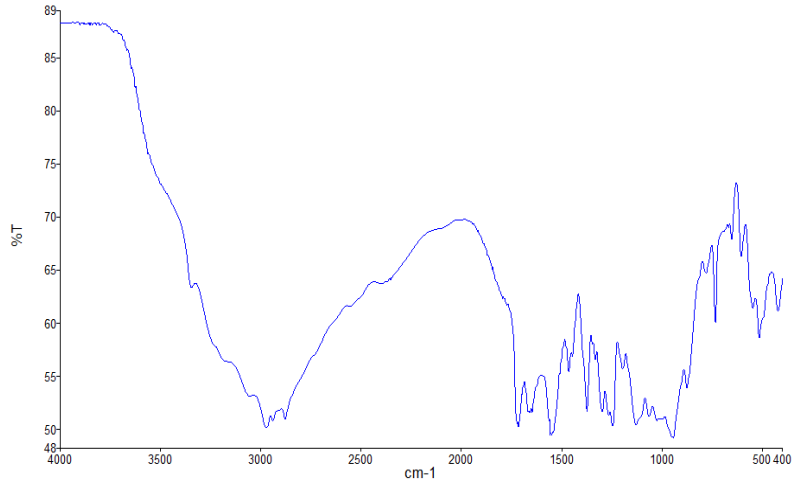

Figure 2. FT-IR spectra of OP – *standard substance* – in KBr-disk.

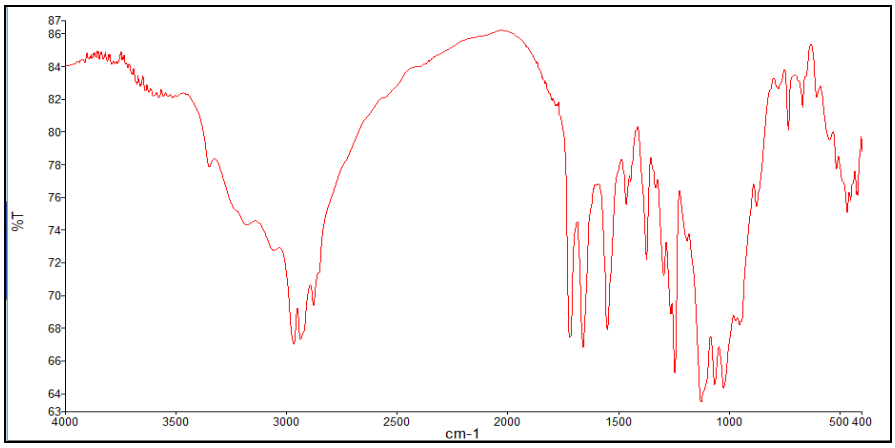

Figure 3. FT-IR spectra of pharmaceutical products TAMINIL N – in KBr-disk.

It is interest to note that in the fingerprint region there are no significant differences between the spectra for KBr disk method.

In PCR and PLS2, the spectra are modeled by one set of factors and each property is modeled by relating the concentration values to those factors. In PLS1, the spectra are modeled by a different set of factors for each property and the concentration values are modeled by the respective factors. Hence PLS1 really contains *n* separate calibrations, where *n* is the number of properties in the method.

The calibrations of this study were carried out with the use of the 'expert' option. The range used was between 4000 - 400 cm^{-1}. In these cases no blanks were selected. Finally, the results are very similar, as can be seen in Table 1. We suggest the use of the PLS2 method, because the peak to peak error value must be maximum five times bigger than root mean square (RMS) error value.

Table 1. Comparison of the OP determination in tablets using FT-IR chemometric approaches.

	TAMINIL N	
	PCR+	PLS2
Content (mg/tablet)	103.44	103.13
RSD (%) (n=5)	3.75	2.06
R M S	0.01428	0.01622
Peak to peak	0.07286	0.07359

Beer-Lambert law was also used for the quantitative determination of OP in pharmaceutical products, but the measurements, could not be performed because we do not find a common baseline between the spectra. As can be seen in Table 1, the results are statistically similar, and we suggest the use of the PLS2 method, because of the smaller value of RSD (< 3.0%).

4. CONCLUSIONS

FT-IR spectrometry is applied for the analytical quantification of **OP** in pharmaceutical formulations using commercial software involving chemometric approaches. The proposed method is simple, precise and not time-consuming compared to the chromatographic methods that exist in literature. Quantification could be done in about 10 -15 minutes, including sample preparation and spectral acquisition.

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