ANALYSIS OF DRUG RELATED IMPURITIES BY INFRARED SPECTROMETRY IN THE CLASS OF STATINS

SZILÁRD ZSOLT FARKAS, SILVIA IMRE*, DANIELA-LUCIA MUNTEAN, AMELIA TERO-VESCAN

Faculty of Pharmacy, University of Medicine and Pharmacy Tîrgu Mureş, Gheorghe Marinescu street 38, 540139
**corresponding author: silsta@yahoo.com*

Abstract

The aim of this study was to determine the applicability of IR spectrometry in the study of drug-related impurities, method which is usually applied as an identification method. We intended to determine the extent to which an impurity of the drug-substance can be detected by IR spectra interpretation and by other possibilities offered by the software used for data processing. Also by choosing proper impurities, impurity A for simvastatin and impurity H for atorvastatin, we aimed to develop a rapid method suitable for stability studies. In order to determine the limit of an impurity detectable by FTIR, mixtures with different concentrations of the drug substance and impurity were analyzed. Confirmation of the exact concentration of the impurity in samples was carried out by high performance liquid chromatography with UV detection.

Rezumat

Lucrarea de faţă şi-a propus să determine aplicabilitatea spectrometriei IR în studiul impurităţilor înrudite chimic, metodă care în prezent este aplicată în laboratoare în specialca metodă de identificare. Ne-am propus să determinăm limita la care o impuritate poate fi detectată din substanţă, prin interpretarea spectrelor şi prin aplicarea posibilităţilor oferite de programul informatic utilizat. Totodată, prin alegerea corectă a impurităţilor, impuritatea A în cazul simvastatinei şi impuritatea H în cazul atorvastatinei, s-a urmărit dezvoltarea unei metode potrivite pentru studii de stabilitate ale acestor substanţe. Pentru determinarea limitei la care o impuritate poate fi detectată, s-au analizat probe marcate cu impurităţi în diferite concentraţii. Confirmarea concentraţiilor probelor în impurităţi s-a realizat prin analiză HPLC cu detecţie în UV.

Keywords: statins, chemically related impurities, IR spectrometry

Introduction

In order to assure effective and safe therapy is very important to identify and quantify the impurities of raw materials, therefore control of impurities is a key component of the quality of pharmaceutical substances and their products and represent one of the biggest challenges for analysts in the industry [17].

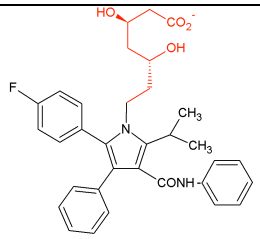
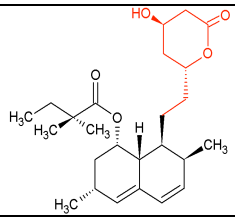
Determination of chemically related substances of active pharmaceutical ingredients is particularly important, especially for medicines used to treat chronic diseases on long term, because a long exposure of the body to impurities, even at low concentrations, can have harmful effects on the organism. Since for the majority of the impurities there are no studies on their toxicity, determination of impurities is an even more critical step of quality control. Understanding the source, control and monitoring of impurities is essential to produce active substances of high-quality.

Because of proven efficacy and safety [15], from their appearance statins have been used as the main treatment used in correcting hypercholesterolemia, so statins also play an increasingly important role in reducing cardiovascular risk in patients with relatively normal plasma cholesterol [16]. Currently there are seven statins marketed worldwide, all seven being considered safe and well tolerated by the body. These considerations were maintained even after the withdrawal from the market of cerivastatin in 2001 by Bayer manufacturing company due to its high toxicity [8].

In the chemical structure of statins, three parts which are essential for their biological action can be identified: a rest of dihydroxy-heptanoic acid (lactone or salt) - is an analogue of the target substrate of the HMGR enzyme; hydrophobic core – with role in the powerful binding between the statin and the HMGR enzyme; lipophilic substituents on the hydrophobic core – with influence on the solubility and therefore on pharmacokinetic properties [11].

Chemical structures of the analyzed statins are presented in table I. In nanomoleculare concentrations statins bind to HMG-CoA reductase, which leads to dislocation of HMG-CoA which is the natural substrate of this enzyme. This competitive inhibition prevents the conversion of HMG-CoA to L-mevalonat, preventing the cascade of cholesterol biosynthesis. Low levels of intracellular cholesterol activate binding proteins responsible for binding of components to sterol, leading to increased gene transcription coding LDL receptor and, therefore, the expression of this receptor on the surface of cells. This leads to further reduction of blood LDL-C by capturing by the liver, which is an LDL receptor mediated effect. Thus, cholesterol - including LDL fraction - is reduced due to two mechanisms: reduced synthesis and increased uptake from the circulation [1].

Table I
Chemical structures of studied statins

No.	Name, abbreviation	Chemical structure
1	Atorvastatin ATO	
2	Simvastatin SIM	

Drugs impurities analysis is mainly performed by chromatographic methods, especially by high-performance liquid chromatography (HPLC) [6,9].

IR spectroscopy has a great potential in elucidating the molecular structure. IR spectrum of an organic substance is like a fingerprint and allows to identify a substance by the comparison with previously recorded reference spectra or to put in evidence important structural parts of the molecules with intense vibration bands [3,4,14] even if the substance is in mixtures or complexes [2]. It is not a common technique for impurities analysis, but it could offer, in certain circumstances, a rapid solution to appreciate the purity of the raw materials. For thus, the impurities of two statins, simvastatin (SIM) and atorvastatin (ATO), were studied in order to determine the aplicability of FTIR spectroscopy in assessing the purity of the drug substances. At the same time we looked forward to assess the level of degradation of materials analyzed; in this respect, the main degradation products of the substances were chosen as indicators of stability, reported in the literature, namely simvastatin acid (impurity A) and atorvastin lactone (impurity H). The main steps were to identify the characteristic bands of simvastatin, atorvastatin and their impurities, and determining the lowest concentration at which an impurity is still detectable in the presence of the main substance. Bands assigned to groups of interest were identified using tables containing the IR vibrational frequencies of groups of atoms [10]. For a precise quantification of the impurity level in tested samples, HPLC analysis was used.

Materials and Methods

Materials

Solvents used for HPLC analysis were HPLC grade produced by Merck. For sample preparation to FTIR analysis potassium bromide for spectroscopy produced by Thermo Electron was used. Standard substances for statins and their main impurities were kindly supplied by Gedeon Richter Romania: atorvastatin calcium; impurity H of atorvastatin (Imp H); simvastatin; impurity A of simvastatin (Imp A).

Instruments

IR analysis was carried out on a Nicolet 380 FTIR spectrometer from Thermo Electron Corporation, HPLC analysis was performed on an Agilent Technologies 1100 series chromatographic system.

Methods

As IR sampling technique the classic method of alkali halide pellet was chosen. The technique involves mixing the solid-state sample of interest with an alkali halide at a 1-2% sample/alkali halide ratio. The mixture is pulverized into a finely homogenous mixture and compacted into a transparent disc [13]. Initially, samples of pure substances were mixed in a mortar of agate with impurities in different ratios, so that the range of concentrations includes the limit allowed by pharmacopoeias [7,19]. This method of mixing two solid-state substances does not assure perfectly homogenous samples, it can lead to errors so, in order to eliminate this error, and concentrations of the samples were confirmed by HPLC analysis.

HPLC methods were previously developed under author's coordination and both were carried out in reverse phase with UV detection [5,18]. Results obtained by HPLC analysis were used in further discussions. The HPLC conditions were as follows: a) for simvastatin: column Luna C 18(2), 3µm, 150x4.6mm (Phenomenex); mobile phase - phosphoric acid 15 mM (A) and acetonitrile (B), gradient elution 0-3 min, 56% A, 3.1-20 min, 56% → 32% A, 20-30 min, 32% A, 30-30.1 min, 32→5% A, 30.1-43 min, 5% A, 43-43.1, 5→56%, 43.1-50 min, 56% A; mobile phase flow 1.6 mL/min; column temperature 20ºC; autosampler temperature 20ºC; wavelength detection 238 nm; injection volume 20 µL. b) for atorvastatin: column Extend C 18, 5µm, 250x4,6mm (Agilent); mobile phase: potassium monophosphate 25 mM (pH=4.3) (A), acetonitrile (B), gradient elution 0-45 min, 75%A, 45-45.1 min, 75→40% A, 45.1-50%, 40%A, 50-50.1, 40→30%A, 50.1-60 min, 30%A, 60-60.1 min, 30-75%, 60.1-75 min, 75%; flow 1 mL/min; column temperature 30ºC; sample temperature 15ºC; detection wavelength 215 nm; injection volume 20 µL.

Results and Discussion

Study of SIM and impurity A

The HPLC method has been developed in order to distinguish chromatographically simvastation from its 6 impurities. Results of HPLC analysis (Figure 1) were calculated using the calibration curve obtained with 7 standard solutions: 0.1-25 μg/mL, Area=39.77c-2.78, R^2>0.99998, RSD=2.58%. The impurity A concentrations in samples are presented in table II.

Table II

Content of Impurity A in SIM samples obtained by HPLC analysis

Name	Concentration of imp A (%)
Mixture 1	11.80
Mixture 2	10.03
Mixture 3	7.31
Mixture 4	5.85

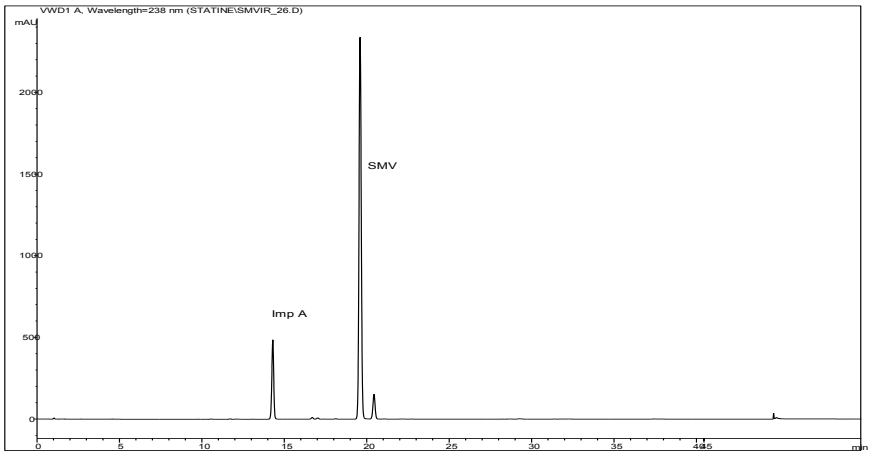


Figure 1

Chromatogram of a SIM – Impurity A mixture

SIM is a very degradable substance, its main degradation product being simvastatin acid (impurity A), that structurally differs from simvastatin by a carboxylic group that produces intense bands on the spectrum, so in mixture it can influence the spectrum of SIM. The permitted limit of impurity A according to EP is maximum 0.4% of the content of active substance.

We identified the characteristic bands in the spectrum of impurity A (Figure 3) that could affect in mixture the spectrum of simvastatin

(Figure 2). There are two bands in the spectrum of impurity A that were not detected in the SIM's spectrum and they are intense enough to be detected in the mixtures' spectra. Regarding the band from 1556 cm^{-1} we can affirm that it is seen significantly up to the 7.31%, after that because of the high signal to noise ratio it isn't considered to have enough intensity for further calculations. Reaching a conclusion about the band from 1718 cm^{-1} is difficult, because in mixtures this band overlaps three of the bands characteristic to SIM. Analyzing these three bands located at 1697, 1710 and 1722 cm^{-1}, respectively, by calculating intensity ratios with a band that is not influenced by the presence of the impurity, we found that as impurity A's concentration decreases in the mixture, the transmittance increases, so there is a continuous relationship between these two aspects, influence that can be observed even at the lowest studied concentration, 5.8%. The comparison of the mixtures spectra with Pharmacopoeia reference spectrum [7], showed that the three bands located in the range of vibration frequencies of C = O ester and lactone (1750-1690 cm-1), X, Y, Z - rated in order of decreasing wavenumbers - overlapping and their intensities are in the relationship X<Y<Z, in the mixture the middle band Y becomes more intense in relation to the other two (Figure 4), as the impurity A is increasing in concentration and in samples mixed in 1:1 ratio, X and Y bands disappear under the intense band at 1718 cm^{-1} characteristic for impurity A.

Essential FTIR software [20] used to process the spectra provides the possibility of comparison and offers numerical results about the similarity of spectra (Table III).

Table III

Degree of similarity between samples spectra and reference spectra

Reference spectra	**Degree of similarity**			
	M 1	M 2	M 3	M 4
SIM	0.922	0.946	0.969	0.974
Imp A	0.534	0.505	0.498	0.496

The obtained results show, as it was expected, that as the impurity concentration in sample decreases, the degree of similarity increases for SIM and vice-versa.

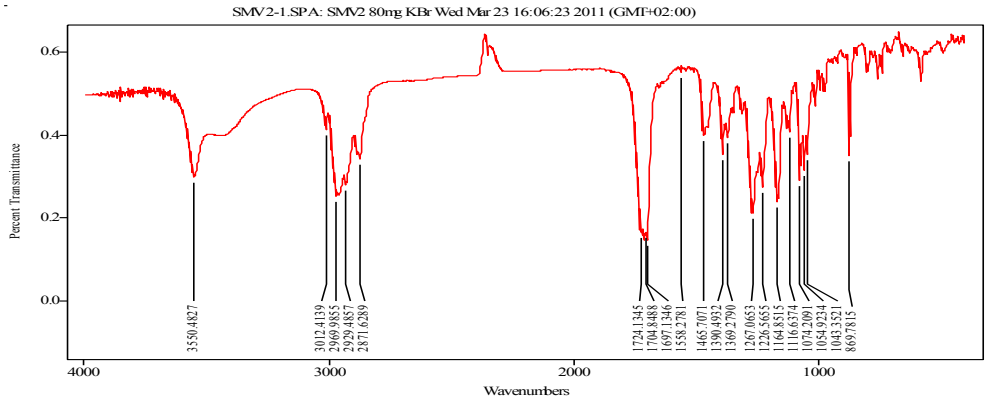


Figure 2
SIM reference spectrum

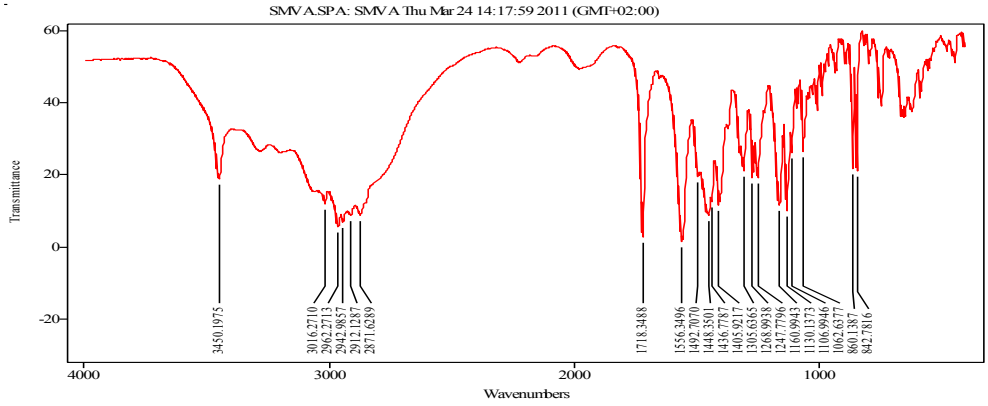


Figure 3
IR spectrum of Impurity A

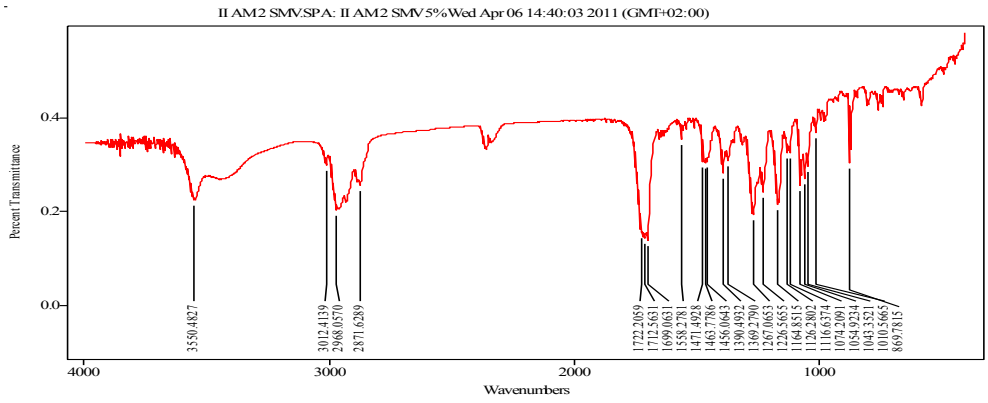


Figure 4
IR spectrum of the mixture with 10% Impurity A

Study of ATO and impurity H

Recent stability studies showed that lactone impurity, called the impurity H, is a product of degradation [12], therefore we chose to study the influence of this impurity on the IR spectral characteristics of ATO. In order to obtain the exact concentrations of samples we applied an HPLC method previously developed in our department (Figure 5) which allows the chromatographic screening of ATO and four of its impurities. Obtained concentrations are presented in table IV by applying the external standard method with a standard solution of 5 µg/mL.

The difference between the two structures, ATO and its lactone impurity, is similar to that found at SIM, but in this case the carboxyl group of ATO is transformed into lactone. The carboxylic group causes a more intense band due to a higher absorption coefficient, so we anticipated that changes in mixtures' spectra will not be as interesting as those found in SIM.

Table IV

Impurity H concentrations in ATO samples obtained by HPLC analysis

Name	Concentration of imp H (%)
Mixture 1	6.33
Mixture 2	1.48
Mixture 3	0.81
Mixture 4	0.30

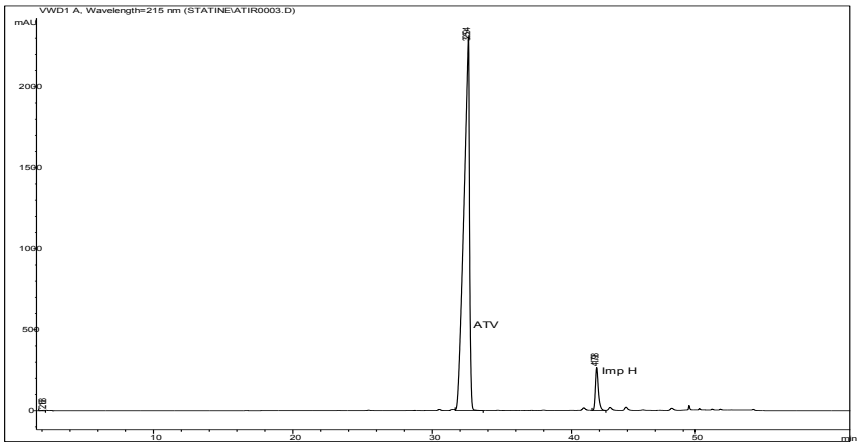


Figure 5

Chromatogram of ATO – Impurity H mixture

In the spectrum of impurity H (Figure 7) at wavenumbers 1716 and 1733 cm^{-1} two bands of medium intensity can be seen, which do not appear in the spectrum of pure atorvastatin (Figure 6). These two bands can be

observed in the mixtures' spectra (Figure 8), to a concentration of 6.33%, after that, because of the noise, they are not significant. We identified in the spectrum of the impurity a band at 1076 cm^{-1} which is attributed to the lactone group. If we follow the variation of intensity of this band in relation with the band from 1110 cm^{-1} in the mixtures of decreasing concentrations in impurity H, there is a significant change in the relationship between them. From the mixture of 6.33%, when the intensity of the two bands is almost equal, gradually to 0.30% concentration, the band 1076 cm^{-1} has a lower intensity, indicating the presence of impurity with decreasing concentrations.

Similar to the case of SIM, we used the possibility of spectra comparison to obtain numerical data for proving results of the spectra interpretation. Results are presented in table V.

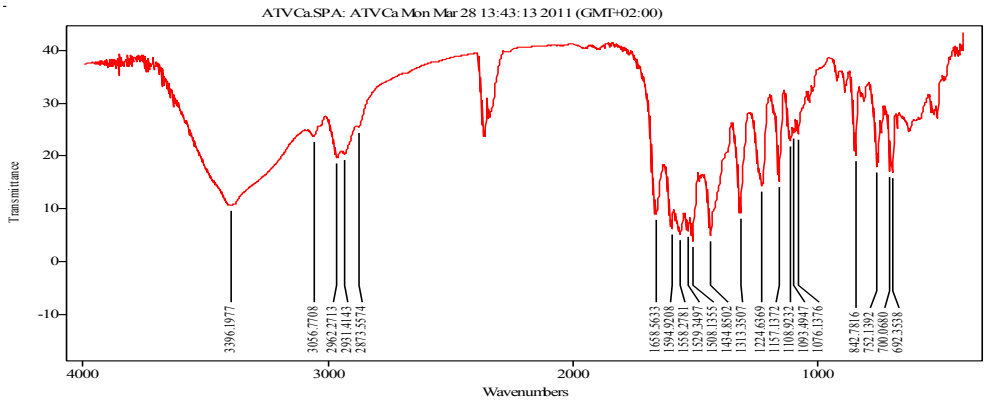


Figure 6
ATO IR reference spectrum

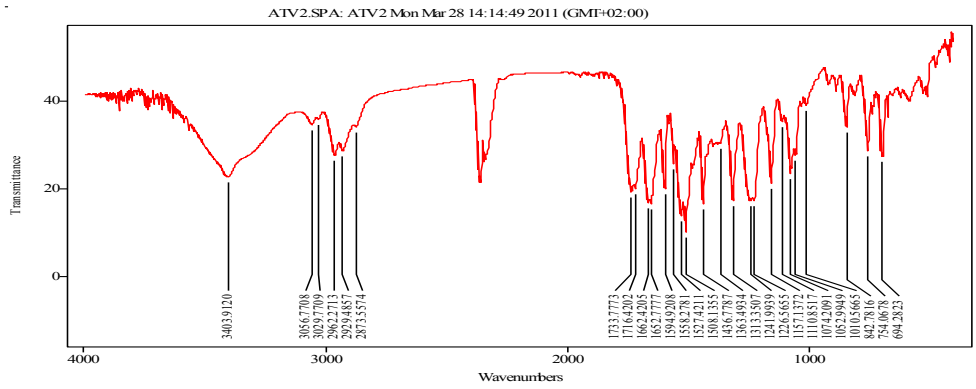


Figure 7
Imp H reference spectrum

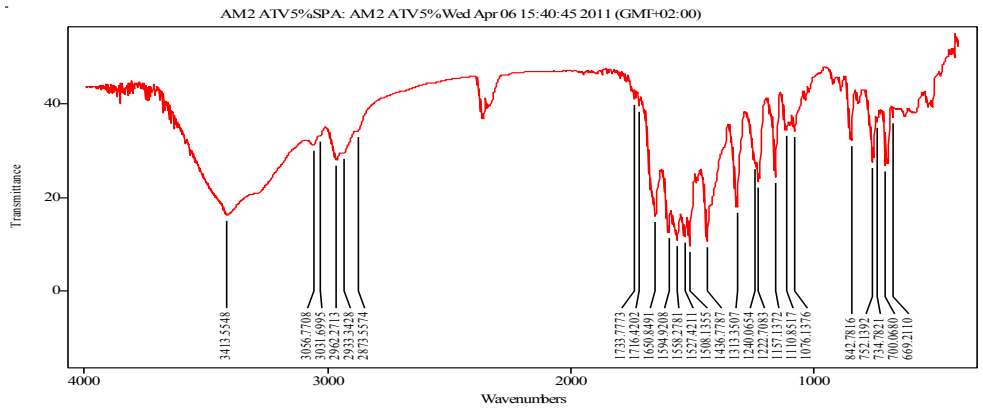


Figure 8

The IR spectrum of the mixture of ATO with 6.33% Imp H

Table V

Spectra comparison results for ATO

Reference spectra	Degree of similarity			
	AM 1	AM 2	AM 3	AM 4
ATO	0.937	0.945	0.946	0.973
Impurity H	0.816	0.804	0.787	0.779

The same normal tendency was observed, as impurity concentration in sample decreases, the degree of similarity increases for ATO and vice-versa.

In conclusion, by choosing correct vibration bands, meaning intense and specific enough, the IR spectra analysis could be a rapid solution to test the degradation of raw materials of pharmaceutical substances. Of course, it is a prerequisite that the impurities of pharmaceutical substances are known and there is one major impurity which could be found.

Conclusions

FTIR spectrometry can be applied to determine the presence or absence of chemically related impurities in raw pharmaceutical substances if their chemical structure is known and are found above a certain limits of percentage in the substance. Concentrations of studied mixtures were chosen including the upper limit accepted by pharmacopoeias. The presence of chemically related impurities, which differ from the substance of interest by a component with high absorption coefficient, can be determined, in certain cases, at concentrations under the pharmacopoeias limits. In the case of ATO we identified differences in the intensity of bands of interest at concentrations allowed by pharmacopoeias, this not being possible for SIM, where the lowest concentration of impurity was too high to draw a conclusion for the limits of pharmacopoeias. However, the intensity ratio of

the three bands located in the range of vibration frequencies of carbonyl from ester and lactone group could serve as a key element for SIM degradation. The possibility of comparing spectra offered by the software used for processing data provided numerical information on the degree of similarity of the spectra, which could serve as an initial sign of the presence of impurity.

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