OPEN ACCESS

GAVIN PUBLISHERS

Advances in
Analytical and Pharmaceutical Chemistry

Research Article

Pedroso TM and Salgado HRN. Adv Anal Pharm Chem: AAPC-103.
DOI: 10.29011/ AAPC-103. 100003

Green Alternative Using Infrared Spectroscopy as an Efficient and Stable Analytical Method for Quantifying Ertapenem Sodium

Tahisa M. Pedroso, Hérida Regina Nunes Salgado*

Department of Pharmaceutics, São Paulo State University, School of Pharmaceutical Sciences, Araraquara, Brazil

***Corresponding author:** Hérida Regina Nunes Salgado, São Paulo State University, Department of Pharmaceutics, Araraquara, Brazil. Tel: +551633016967; Fax: +551633016960; Email: salgadoh@fcfar.unesp.br

Citation: Pedroso TM, Salgado HRN (2018) Green Alternative Using Infrared Spectroscopy as an Efficient and Stable Analytical Method for Quantifying Ertapenem Sodium. Adv Anal Pharm Chem: AAPC-103. DOI: 10.29011/ AAPC-103. 100003

Received Date: 28 June, 2018; **Accepted Date:** 05 July, 2018; **Published Date:** 13 July, 2018

Abstract

An eco-friendly method by Fourier-Transform Infrared transmission spectroscopy for quantifying ertapenem sodium was developed. The method, conducted in potassium bromide pellets containing ERTM, was analyzed. The method involved spectral analysis by absorbance band measurements corresponding to one of the carbonyl groups. This method was validated according to the International Conference on Harmonization guidelines. The linearity range was found to be 0.6 to 1.6 mg g^{-1} (regression equation: $y = 0.5141x + 0.021$, $r^2 = 0.9993$). The environmentally friendly method is very useful for the routine quality control of ertapenem sodium. The highly efficient technique allows the characterization and quantification of ERTM, without using any organic solvent, because samples are prepared in potassium bromide as unique reagent. Therefore, this new validated method can contribute to the reduction of organic solvent waste from the chemical and pharmaceutical industry and, as a result, the impact of its activities on the environment is high relevance.

Keywords: Environmentally Friendly; Ertapenem Sodium; Green Chemistry; Infrared Spectroscopy; Method Validation

Introduction

Antibiotics are essential for the treatment of many diseases and increased development of bacterial resistance due to beta-lactamases extended or carbapenemases spectrum is of concern. Carbapenems still assume a great role in the treatment of serious infections because among the β-lactams currently available, they are relatively resistant to hydrolysis by most β-lactamases and still target penicillin binding proteins. This class of antibiotics are considered as drug of choice in many multidrug resistant infections [1].

Carbapenem consists in a group antibiotic with similar spectrum activity with little differences in activities of individual agents. Ertapenem sodium (Figure 1) is parenteral-β -methyl carbapenem developed in 2001. It is broad-spectrum β-lactam antibiotic with the bactericidal property which differs from other members of this class by the presence of the meta-substituted benzoic acid substituent, this moiety increases the lipophilicity of ertapenem that is approximately 94% protein bound, the long half-life gives it the advantage of once daily dose regimen [2].

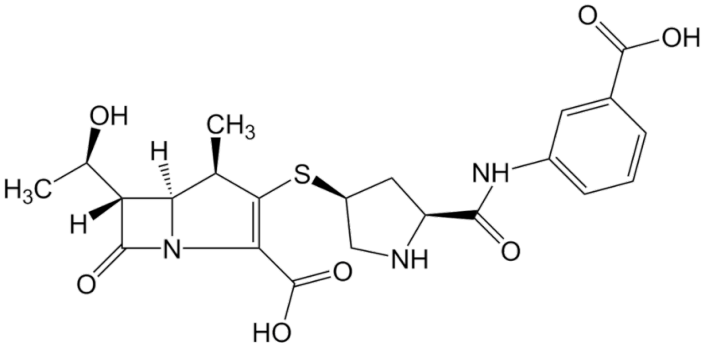

Figure 1: Chemical structure of Ertapenem sodium (CAS 153773-82-1).

Infrared spectroscopy is an excellent method for the identification of organic functional groups and has currently been successfully used for quantification of substances. The academic and industrial researches aim to reduce the environmental impacts and they must seek alternatives to reduce, prevent or eliminate chemical residues in their routine processes. Infrared spectroscopy can be a good option because it does not use organic solvents. Our results confirmed the potential of this green method for routine

applications [3-7].

A review on the analytical methods was performed and no analytical method was found to quantify ertapenem by infrared [8]. In the present study, we have used samples that were prepared in potassium bromide (KBr). To develop and validate a method of analysis for quantifying ertapenem, pellets containing different concentrations were analyzed by Fourier-Transform Infrared (FTIR) transmission spectroscopy. This technique does not need to use organic solvents commonly used in traditional methods. This novel approach could prove to be of considerable utility for the analysis of pharmaceutical formulations to assure the quality and efficacy of ertapenem sodium.

Experimental

Chemicals

The chemicals used were ertapenem sodium 99.2% (lot EB004C1) and ertapenem sodium in lyophilized injectable containing 1 g of the active pharmaceutical ingredient (API) (lot 2178140) both kindly donated by Merck Sharp & Dohme™ and potassium bromide was used to make the pellets (analytical reagent, Synth™).

Preparation of Pellets

Performance: A dilution of 1:100 with potassium bromide previously dried in the oven at 105°C and ertapenem sodium from a pool of five sample vials. To make the pellets, 1 mg of ertapenem sodium (100 mg of the 1:100 dilution in potassium bromide) was added in 100 mg of potassium bromide. Both were mixed and pulverized on an agate mortar for homogenization. Then, the mix was placed in a mold and was pressed under vacuum 80 pressure kN, forming a transparent pellet with a weight of 200 mg. The same procedure was performed to ERTM reference substance and ERTM in lyophilized powder for injectable solution. The reading was held in transmittance to IR Solution software. The spectral region corresponding to one of the carbonyl groups in the ERTM molecule (1720-1800 cm^{-1}), was measured quantitatively. The infrared spectra were recorded with 40 scans at a resolution of 4.0. The determinations were performed in triplicate.

Equipment

Spectra of potassium bromide (KBr) pellets containing ERTM was obtained using the FT-IR spectrometer Shimadzu™ (Kyoto, Japan), model IR Prestige-21 and IR solution software, analytical balance model H51Mettler Toledo and an oven model 702.780 (Quimis, Brazil) to dry the KBr until constant weight.

Method Validation

This method was validated according to the International Conference on Harmonization guidelines [9] for linearity, selectivity, accuracy, precision, robustness, detection limit and quantification limit.

Linearity

The linearity was evaluated by regression analysis of ertapenem sodium standard mixture in six points at concentration range (0.6 to 1.6 mg) prepared on three consecutive days (n = 3). The regression lines were calculated by the least-squares method. Statistical evaluation was made by ANOVA. The values were reported as the average ± S.D. of the calibration curves.

Precision

Repeatability (Intra-day precision) and intermediate precision (inter-day precision) were carried out according international guidelines [9]. The repeatability was studied by the performance of seven determinations of the sample on the same day and identical working conditions. Intermediate precision was assessed by performing the assay for a second analyst and in three different days under the same experimental conditions. At the end of the test, the relative standard deviation percentage (RSD) values of the determinations were analyzed [9].

Accuracy

Accuracy was achieved via the recovery assay, in which a known quantity of RS was added to a known quantity of the sample [9]. The recovery was performed in the three levels, R1, R2 and R3, and the pellets were prepared according to the Table 1, in triplicate.

	ERTM sample1 (mg)	ERTM RS[1](mg)	KBr[2](mg)	Final concentration (mg/pellet)
Sample	60	–	140	0.6
R1	60	20	120	0.8
R2	60	40	100	1
R3	60	60	80	1.2
RS	–	60	140	0.6

[1]Diluited 1:100 (w/w) in KBr; [2]Sufficient amounts for the preparation of pellets with a total weight of 200 mg; ERTM: Ertapenem sodium; RS: Reference standard.

Table 1: Preparation of pellets for the recovery assay of the method of FT-IR spectroscopy for ertapenem sodium.

The recovery percentage was calculated by the equation determined by the Association of Official Analytical Chemists – AOAC [10].

Robustness

The robustness of the method was evaluated making small alterations to the conditions, to show that the validity of the method is maintained even in small parameter modifications. The following parameters were varied: KBr brand, compression duration and compression pressure. The obtained responses were evaluated according to the R.S.D. among the dosages.

Detection and Quantification Limits

The detection (LOD) and quantification (LOQ) limits were calculated based on the intercept standard deviation and the curve slope, as described in the literature [9]. Three different curves were performed for the obtainment of the necessary data for the calculation. Were LOD = 3.3 (SD/a) and LOQ = 10 (SD/a) (as it is inclination of the analytical curve and SD is intercept standard deviation).

Results and Discussion

Spectroscopy in the infrared is a rapid analysis technique and has been accepted as analytical method due to the numerous advantages, such as being a green technique, decreasing the impact in the environment since it does not use organic solvents and being an excellent alternative for the industry, by reducing or even eliminating the generation of waste chemicals in their routine procedures. Moreover, it is a technique which requires minimal or no sample pretreatment, it also provides accuracy in comparison to other methods and it is possible to detect impurities as well [11,12].

Infrared spectroscopy is based on observation of the vibration of molecules that are excited by a beam of radiation in the infrared zone. Through the examination of the transmitted light, it can be checked that the amount of energy that was absorbed at each wavelength is in the form of characteristic spectra for each substance. With a detailed analysis of absorption bands and compared with spectra of chemical reference, insurance identification data is obtained and, therefore, it can be quantified [13].

The medium infrared spectrophotometers are adapted to the region 4000 cm^{-1} to 650 cm^{-1}, or possibly up to 200 cm^{-1} for recording spectra that are characteristic of the molecule as a whole [14]. Spectroscopy in the infrared medium (4000 to 400 cm^{-1}) is considered a quintessential identification test, being able to differentiate substances with small structural differences and is therefore the most used for identification purposes [13-15].

The absorption spectra in the infrared region, showed characteristic absorption bands of β-lactam substances and are according to the carbapenem nucleus. such as 3500-3300 cm^{-1} (N-H pyrrolidinyl group, stretching vibration), 3100-3000 cm^{-1} (C-N stretching vibration), 3000-2850 cm^{-1} (C-H stretching vibration), 1760-1700 cm^{-1} (C=O carboxylic acid function, stretching vibration), 1680-1630 cm^{-1} (C=O amide group, stretching vibration), 1600, 1580, 1500, 1450 cm^{-1} (C=C aromatic group), 1350-1000 cm^{-1} (C-N stretching vibration), 1640-1550 cm^{-1} (N-H secondary amide, bending vibration). These data were analyzed according to the reference books [13-15].

Besides being used to characterize the molecule, this method was developed to measure the band quantitatively, by corresponding to one of the carbonyl groups in the molecule ERTM, centered in the region between 1720 and 1800 cm^{-1}. The absorption spectra in the infrared region in KBr tablets indicated similar characteristics between the ERTM RS and ERTM lyophilized powder for injectable solution. The adjuvants presented in ERTM lyophilized powder for injectable solution (bicarbonate sodium and hydroxide sodium) did not interfere in the quantitative analysis region as shown in Figure 2.

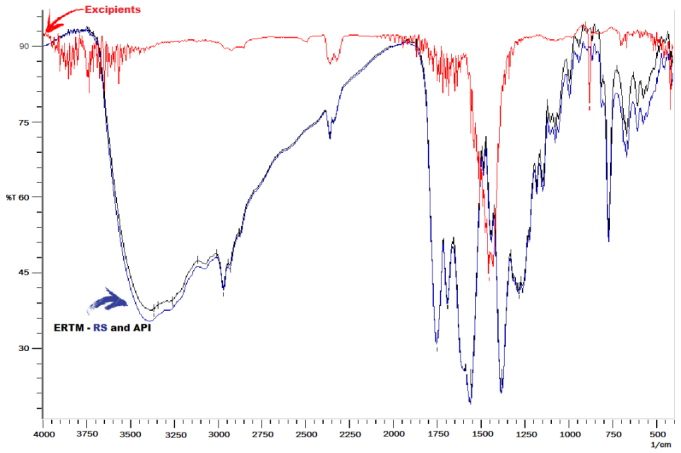

Figure 2: IR spectra obtained from ERTM RS, ERTM in lyophilized powder for injectable solution and excipient.

The methods were validated by following the guidelines for validation of analytical methods, according to the 2005 recommendation International Conference on Harmonization, the 2003 guidelines of Brazil, and FDA, 2000. The analyzed parameters were linearity, Limit of Quantitation (LOQ), Limit of Detection (LOD), precision, accuracy, selectivity and robustness. The analytical curve for ertapenem sodium RSR was built by arranging the average value of absorbance in relation to its respective concentration as shown in Figure 3.

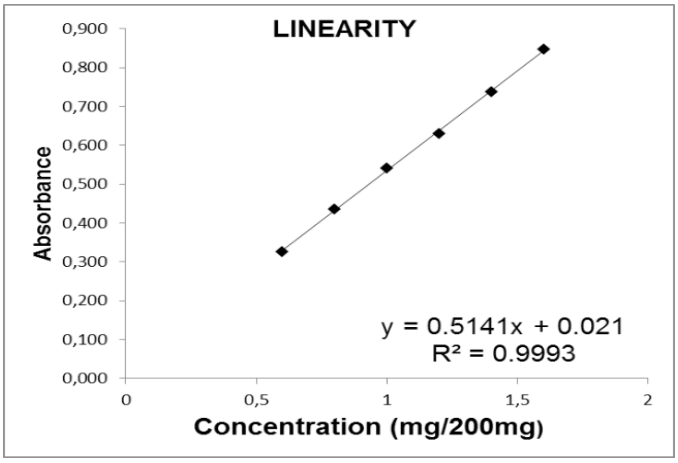

Figure 3: Calibration curve for ERTM by infrared spectroscopic method.

The analytical curve of ERTM RS was evaluated by ANOVA and this is shown in Table 2.

Source of variation	Degree of free dom	Sum of squares	Variability	F calc ulated	F critical
Between conce ntration	5	0.555	0.111	902. 981*	3.106
Linear regr ession	1	0.555	0.555	4508. 424*	4.747
Deviation of linearity	4	0.001	0	1.62	3.259
Residue	12	0.001	0	.....	.....
Total	17	0.557	.....	.....	.....
*Significant at p <0.05 %					

Table 2: Analysis of variance by obtaining the analytical curve of ertapenem sodium RS, using spectroscopy method in the infrared region.

The study of validation results show that the infrared spectroscopy method is appropriated for quantifying ertapenem sodium (Table 3).

Parameters	Results
Content of ERTM	101.82 %
Linearity	y = 0.5141x + 0.021, r^2 = 0.9993 (0.6 to 1.6 mg)
Intra-day precision	RSD = 1.74 %
Inter-day precision	1st day 99.51%; 2nd day 101.20% and 3rd day 101.78% - RSD = 1.17 % 1st analyst 100.59% and 2nd analyst 102.49 % RSD = 1.32 %
Accuracy	97.60 %, RSD = 1.92%
LOD	0.16 mg
LOQ	0.49 mg
Robustness	KBr brand: Synth®-101.78; Vetec®-100.79; RSD 0.69 %
	Compression duration: 9 min, 100.59 %; 10 min, 101.78 %; 11 min, 102.57 % RSD 0.98 %
	Compression pressure: 75 kN, 100.70 %; 80 kN, 101.20 %; 85 kN, 100.24 % RSD 0.48 %

Table 3: Results of Infrared spectroscopy method validation.

In this concept, infrared spectroscopy is an excellent quantitative method which has characteristics to be consider a green analytical method since does not use organic solvents [3-7, 16-18].

The correlation peaks shown in the spectra, using spectroscopy technique in the infrared region, allows us to characterize the analyte. It presents great evidence of identity of a structure being practical, fast and selective, with the advantage of requiring small amounts of sample, having viable budget (referring to instrumentation), increasing the ability to identify or characterize complex structures, not handling toxic materials and not generating waste organic solvents.

This manuscript intends to wake up old chemical concepts aiming a new ecological discussion converging the past and the present to get a better and sustainable quality control in pharmaceutical and chemical industries [19].

There are many techniques that can be used to evaluate quality control of pharmaceutical products. The limitations of this technique should be considered. The sensitivity of this method is about 50-fold less when compared to the HPLC technique presented by us in another work. However, the options involving the ecological conceptions are immediate alternatives which should be adopted by academic and industrial society.

The main contribution of this paper is to present an excellent technique for the identification of substances which can now also be used for the quantification of drugs with the advantage of being "greener" than the traditional techniques. The FT-IR spectroscopy

can be easily applied in routine analysis for the determination and quantification of drugs and have been reported as a good and proper technique by our study group. [6,16]. There is a journey on greener pathways, and we hope these pathways will open up new opportunities for the future [20].

Conclusion

A simple, fast and reproducible infrared spectroscopy method was developed and validated for quantifying ERTM. The technique allows the characterization and quantification of ERTM, with the advantage of not using any organic solvent, hence contributing to the reduction of organic solvent waste from the chemical and pharmaceutical industry and, as a result, the impact of its activities on the environment. Therefore, the results can be applied to the analysis of pharmaceutical formulations to assure the quality and efficacy of ERTM.

Acknowledgements

We are very grateful to the laboratory Merck Sharp & Dohme for donating the drug, and FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil, Project 2013/12959-0 and 2015/03412-3, and CNPq (Brasília, Brazil) for financial support.

References

1. Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA (2011) Carbapenems: Past, Present, and Future. Antimicrobial Agents Chemotherapy 55: 4943-4960.
2. El-Gamal MI, Brahim I, Hisham N, Aladdin R, Mohammed H, et al. (2017) Recent updates of carbapenem antibiotics. European Journal of Medicinal Chemistry 131: 185-195.
3. Moreno AH, Salgado HRN (2012) Development and validation of the quantitative analysis of ceftazidime in powder for injection by infrared spectroscopy. Physical Chemistry 2: 6-11.
4. Tótoli EG, Salgado HRN (2012) Development and validation of the quantitative analysis of ampicillin sodium in powder for injection by Fourier-transform infrared spectroscopy (FT-IR). Physical Chemistry 2: 103-108.
5. Vieira DCM, Salgado HRN (2012) Development and validation of the quantitative analysis of cefuroxime sodium in powder for injection by infrared spectroscopy. Advances in Analytical Chemistry 2: 80-87.
6. Kogawa AC, Salgado HRN (2013) Development and validation of infrared spectroscopy method for the determination of darunavir in tablets. Physical Chemistry 3: 1-6.
7. Kogawa AC, Mello NP, Salgado HRN (2016) Quantification of doxycycline in raw material by an eco-friendly method of infrared spectroscopy. Pharmaceutica Analytica Acta 7: 1-4.
8. Pedroso TM, Salgado HRN (2016) A critical review of analytical methods for Determination of Ertapenem sodium. Critical Reviews in Analytical Chemistry 46: 15-21.
9. ICH - International Conference on Harmonization (2005) Validation of analytical procedures: Text and Methodology Q_2 (R_1). Geneva.
10. AOAC (2002) Official methods of analysis. 17th ed. Association of Official Analytical Chemists. Gaithesburg.
11. Farouk F, Moussa BA, Azzazy HMES (2011) Fourier transform infrared spectroscopy for in-process inspection, counterfeit detection and quality control of anti-diabetic drugs. Spectroscopy 26: 297-309.
12. Corrêa JCR, Salgado HRN (2014) A platform for designing quantitative infrared spectrophotometric method for drugs and pharmaceuticals analysis: a rediscover for an ecological and safer technique in the routine quality control laboratories. World Journal of Pharmacy and Pharmaceutical Sciences 3: 2056-2059.
13. Pavia DL, Lampman GM, Kriz GS, Vyvyan JR (2010) Introdução à Espectrofotometria. 4. ed. São Paulo: Cengage Learning: 700.
14. Farmacopeia Brasileira (2010) Agência Nacional de Vigilância Sanitária, Ed.; 5th ed. Brasília, Brazil: 1444.
15. Silverstein RM, Webster FK, Kiemle DJ (2007) Identificação espectrométrica de compostos orgânicos. 7. ed. Rio de Janeiro: LTC: 490.
16. Marco BA, Salgado HRN (2016) Development and validation of an innovative method for the determination of cefadroxil monohydrate in capsules. Physical Chemistry 6: 67-74.
17. Consortti LP, Salgado HRN (2017) Green method for quantification of sodium cefotaxime in lyophilized powder by infrared spectroscopy. Journal of Pharmaceutical Sciences & Emerging Drugs 5: 1-6.
18. Kogawa AC, Salgado HRN (2017) Spectrophotometry in infrared region: a new, low cost and green way to analyze tablets of rifaximin. Current Pharmaceutical Analysis 13: 1-5.
19. Koenig SG, Sneddon HF (2017) Recent advances in flow chemistry in the pharmaceutical industry. Green Chemistry 19: 1418-1419.
20. Varma R S (2014) Journey on greener pathways: from the use of alternate energy inputs and benign reaction media to sustainable applications of nano-catalysts in synthesis and environmental remediation Green Chemistry 16: 2027-2041.