



Received on 10 February, 2015; received in revised form, 19 April, 2015; accepted, 30 June, 2015; published 01 September, 2015

SIMULTANEOUS SPECTROSCOPIC ESTIMATION AND VALIDATION OF GEMCITABINE AND TAMOXIFEN IN SYNTHETIC MIXTURE BY FIRST ORDER DERIVATIVE METHOD

M. L. Thakkar, D. B. Shah * and D. G. Maheshwari

Department of Quality Assurance and Pharm Regulatory Affairs, L. J. Institute of Pharmacy, Ahmedabad
Gujrat, India

Keywords:

Gemcitabine, Tamoxifen,
UV spectrophotometric method

Correspondence to Author:

Mr. Darshil Shah


Assistant Professor,
L. J. Institute of Pharmacy
Between Sarkhej circle & Katariya
motors, S. G. Road, Ahmedabad-
382210, Gujarat, India.

E-mail: darshilshah89@yahoo.com

ABSTRACT: A novel, simple, rapid and sensitive UV spectrophotometric method has been developed for simultaneous estimation of Gemcitabine and Tamoxifen. Gemcitabine and Tamoxifen show maximum absorbance at 269 nm and 238 nm respectively. The method employs first order derivative method. Here in this method, TAM show zero absorbance i.e Zero Crossing Point (ZCP) at 285 nm and at this wavelength GEM show measureable absorbance and ZCP of GEM is 224 nm and at this wavelength TAM show measurable absorbance. So absorbance of GEM and TAM is measured at 285 nm and 224 nm respectively. Both the drugs obey Beer's Law in the concentration ranges employed for this method. R^2 value of GEM and TAM was found to be 0.996 and 0.998 respectively. Accuracy and reproducibility of the proposed method was statistically validated by recovery studies. Limit of detection and (LOD) and limit of quantification (LOQ) of GEM was found to be 0.02833 and 0.08586 respectively. LOD and LOQ of TAM was found to be 0.09565 and 0.2898 respectively. The method is found to be rapid, precise and accurate and can easily be employed in the laboratory for the routine estimation of drugs.

INTRODUCTION: ¹⁻⁵ Tamoxifen (TAM), (z)-2-[4-(1,2- Diphenyl – 1 - butenyl)phenoxy - N, N - dimethylethanamine is an antagonist of the estrogen receptor in the breast tissue via its active metabolites 4-hydroxytamoxifen. In endometrium, it behaves as agonist, so characterized as selective estrogen receptor modulator.

TAM is also under therapeutic category of bone density conservation agent and antineoplastic agent, hormonal Tamoxifen is endocrine (antiestrogen) therapy for hormone receptor positive breast cancer in pre-menopausal women, and is also strand for post-menopausal. TAM is slightly soluble in water and soluble in methanol, ethanol and acetone.

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.6(9).4000-03</p>
<p>Article can be accessed online on: www.ijpsr.com</p>	
<p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(9).4000-03</p>	

Gemcitabine (GEM), 2'-Deoxy-2',2'-difluorocytidine is an antineoplastic anti-metabolite. GEM is also under therapeutic category of antiviral agent, immunosuppressive agent, enzyme inhibitor and radiation sensitizing agent. GEM was found to be a very potent inhibitor of human leukemia cells grown in culture and is

since been used in the several types of cancer treatment including metastatic breast cancer, non-small cell lung cancer, pancreatic cancer and ovarian cancer. It is used in first line treatment of patients with metastatic breast cancer.

Literature survey reveals several methods that have been used for the quantitative determination of the two drugs individually, such as for Gemcitabine(GEM) UV spectrophotometry, HPLC, estimation in pharmaceutical dosage forms by UV spectrophotometry and RP- HPLC and in plasma determination of GEM by HPLC. Tamoxifen (TAM) is estimated by LC and HPLC with UV detection.

MATERIALS AND METHODS:

Reference standard of GEM is obtained from Intas Pharmaceuticals, Bavla and TAM was obtained from Mascot Industry PVT LTD, Naroda. Methanol is from Samir Tech-Chem PVT LTD, Vadodara. Shimadzu UV-1800 Spectrophotometer with 10mm matched quartz cells was used for experiment. Absorption and over lain spectra were recorded over the wavelength range 200-400 nm, using 1cm quartz cells at a scan speed fast and fixed slit width of 1.0 nm.

Experimental:

Preparation of standard stock solution:

Stock solutions (1000 μ g/ml) of GEM and TAM were prepared by dissolving separately 100 mg of drug in 100 ml of methanol. The stock solution were suitably diluted to produce solution of concentration 100 μ g/ml, and further dilution was done to produce conc. of 10 μ g/ml, these working solution was scanned in the entire UV range(200-400 nm) to determine the maximum wavelength. Absorption maxima of GEM and TAM were detected at 269 nm (λ_1) and 238 nm (λ_2), respectively and overlain spectra was recorded. A series of standard dilutions of each drug were prepared having concentration range of 5-25 μ g/ml. Both TAM and GEM showed linearity with absorbance in the range 5-25 μ g/ml at their respective maxima. The absorbance was measured at 285 nm for GEM and 224 nm for TAM and calibration curves were plotted at these wavelengths.

Preparation of synthetic mixture:

TABLE 1: INGREDIENT OF SYNTHETIC MIXTURE

	Names	Quantity (mg)
Drug	Gemcitabine	10
	Tamoxifen	10
Excipients	Cross-carmellose Sodium	5
	Talc	1.25
	Magnesium Stearate	1.25
	Povidone	6.25
	Microcrystalline cellulose	22.5
	Lactose monohydrate	16.25

Recovery studies:

Recovery studies were done so as to check the accuracy of the method. The accuracy of the method was assessed by taking known amounts of TAM and GEM in standard mixture solution and absorbance was determined at 224 nm and 285 nm respectively. Concentration of the drugs in the mixture was calculated using the equations. The analysis was done in a set of 3 replicates.

Repeatability:

Repeatability is given by interday and intraday precision. The assay and recovery procedures were repeated for three times, on the same day and once for three successive days for both the drugs. Repeatability and Interday precision show in Table 2 & 3 respectively.

TABLE 2: REPEATABILITY STUDY FOR GEM AND TAM FOR 10 μ G/ML

Sr. No	Gemcitabine (GEM)	Tamoxifen (TAM)
	Absorbance at 285 nm	Absorbance at 224 nm
1	0.03404	0.01725
2	0.03398	0.01721
3	0.03405	0.01724
4	0.03404	0.01719
5	0.03397	0.01721
6	0.03401	0.01722
Mean	0.034015	0.01722
SD	0.0000339116	0.0000219089
%RSD	0.0996961	0.127229

TABLE 3: INTERDAY PRECISION FOR GEM AND TAM FOR 10 μ g/ml

Sr.No	GEM	TAM
1	9.81	9.9
2	9.84	10.1
3	9.78	10.6
Mean	9.81	10.02
%Estimated	98.1	100.2
SD	0.03	0.10583
%RSD	0.03058	0.10561

RESULTS:

Absorption maxima:

Absorption maxima of GEM and TAM were detected at 269nm (λ_1) and 238nm (λ_2), respectively and overlain spectra was recorded (Fig. 1).

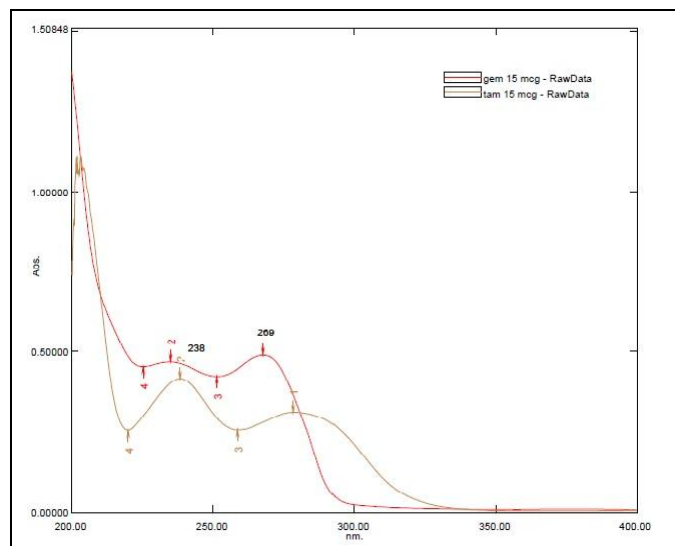


FIG.1: OVERLAIN SPECTRA OF TAM AND GEM ZERO CROSSING POINT (ZCP)

GEM and TAM show zero absorbance at wavelength 224 nm and 285 nm respectively and

TABLE 4: OPTICAL CHARACTERISTICS

Sr.No	Characteristics	GEM	TAM
1	λ_{max}	269 nm	238 nm
2	Beer's Law limit ($\mu\text{g/ml}$)	5-25 $\mu\text{g/ml}$	5-25 $\mu\text{g/ml}$
3	ZCP	224 nm	285 nm
4	Regression equation	$Y = -0.004x + 0.0035$	$Y = -0.000796x + 0.0090$
I	Slope	-0.004	-0.000769
II	Intercept	0.0035	0.0090
III	R^2	0.996	0.998
IV	LOD ($\mu\text{g/ml}$)	0.02833	0.09565
VI	LOQ ($\mu\text{g/ml}$)	0.08586	0.2898

Recovery studies:

Recovery studies were done so as to check the accuracy of the method. The analysis was done in a

first order derivative overlain spectra of GEM and TAM at 20 $\mu\text{g/ml}$ was recorded (Fig. 2).

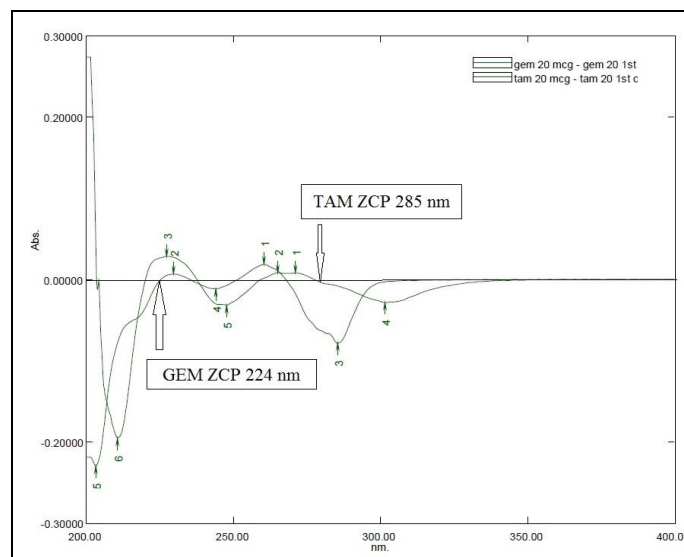


FIG.2: FIRST ORDER OVERLAIN SPECTRA OF TAM AND GEM

set of 3 replicates and results are summarized in Table 5. Recovery was close to 100% stating the accuracy and reproducibility of the method.

TABLE 5: RECOVERY STUDIES

Drug in standard mixture solution ($\mu\text{g/ml}$)		%Recovery $\pm\text{SD}$		%RSD	
GEM	TAM	GEM	TAM	GEM	TAM
18	18	99.53 \pm 1.42	100.33 \pm 1.53	1.433	1.527
20	20	99.61 \pm 1.83	100.3 \pm 1.34	1.84	1.341
22	22	100.31 \pm 1.75	99.8 \pm 1.62	1.753	1.63

DISCUSSION: The validation parameters were studied at both the wavelengths for the method. Accuracy and reproducibility was determined by calculating the recovery that was close to 100%. Precision was calculated as repeatability (SD and

%RSD). The proposed method is simple, precise, accurate and reproducible. Due to high sensitivity and simple sample preparation, the method can be used for routine analysis.

CONCLUSION: Simultaneous estimation of GEM and TAM is performed by first order derivative method and method is validated according to ICH Q2 (R1) guideline.

ACKNOWLEDGEMENTS: The authors are thankful to Dr. K. Pundarikakshudu, Director of L. J. Institute of Pharmacy, Ahmedabad, India for providing all the facilities and encouragement to carry out the work.

REFERENCES:

1. Williams N, Bulstrode C, O'Connell P: Short Practice of surgery. Hodder Arnold Publication, Twenty sixth Revised Edition 2013.
2. Drug bank [Internet], Drug introduction [cited 2015 Jan 3] Available from: www.drugbank.ca/drugs/db00675
3. Drug bank [Internet], Drug introduction [cited 2015 Jan 3] Available from: www.drugbank.ca/drugs/db006441
4. Rx list [Internet], Drug introduction [cited 2015 Jan 3] Available from: www.rxlist.com/gemzar-drug.htm
5. Beckett AH, Stenlake JB: Practical Pharmaceutical Chemistry. CBS Publishers, Second Edition 2001.
6. Chatwal GR, Anand SK: Instrumental methods of chemical analysis. Himalaya Publishing House PVT LTD, Mumbai, Edition 5, 2012: 2.149-2.184.

How to cite this article:

Thakkar ML, Shah DB and Maheshwari DG: Simultaneous Spectroscopic Estimation and Validation of Gemcitabine and Tamoxifen in Synthetic Mixture by First Order Derivative Method. Int J Pharm Sci Res 2015; 6(9): 4000-03. doi: 10.13040/IJPSR.0975-8232.6(9).4000-03.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)