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First derivative spectrophotometric determination of granisetron hydrochloride in presence of its hydrolytic products and preservative and application to pharmaceutical preparations

Ismail I. Hewala,[a*] Mona M. Bedair[b] and Sherif M. Shousha[c]

Granisetron is a selective 5-HT$_3$ receptor antagonist used in prevention and treatment of chemotherapy-induced nausea and vomiting. The drug is available in tablet dosage form and parenteral dosage form containing benzyl alcohol as a preservative. The main route of degradation of granisetron is through hydrolysis. The present work describes the development of a simple, rapid, and reliable first derivative spectrophotometric method for the determination of granisetron in presence of its hydrolytic products as well as the formulations adjuvant and benzyl alcohol. The method is based on the measurement of the first derivative response of granisetron at 290 nm where the interference of the hydrolytic products, the co-formulated adjuvant and benzyl alcohol is completely eliminated. The proposed method was validated with respect to specificity, linearity, selectivity, accuracy, precision, robustness, detection, and quantification limits. Regression analysis showed good correlation between the first derivative response and the concentration of granisetron over a range of 8–16 μg ml^{-1}. Statistical analysis proved the accuracy of the proposed method compared with a reference stability indicating high performance liquid chromatography method. The described method was successfully applied to the determination of granisetron in different batches of tablets and ampoules. The assay results obtained in this study strongly encourage us to apply the validated method for the quality control and routine analysis of tablets and parenteral preparations containing granisetron.

Keywords: granisetron; hydrolytic degradation; first derivative; spectrophotometry; pharmaceutical preparations

Introduction

Intravenous chemotherapy in patients suffering cancer sometimes requires the co-administration of several medications to prevent the severe side effects that are enough to deter patients from continuing therapy.[1] Granisetron hydrochloride (GRS), 1-Methyl-N-[(1R, 3r, 5S)-9-methyl-9-azabicyclo (3.3.1) non-3-yl]-1H-indazole-3-carboxamide hydrochloride is a selective 5-HT$_3$ receptor antagonist used in prevention and treatment of chemotherapy-induced nausea and vomiting.[2] GRS is available as tablets and parenteral solutions, which are formulated as single-dose preservative-free injections and multi-dose vials that contain benzyl alcohol as preservative.[3] The literature reveals that several methods have been reported for determination of GRS in pharmaceutical preparations and biological fluids. These include high performance liquid chromatography (HPLC) using DAD (diode-array detection),[4] fluorescence,[5–11] ultra-violet (UV),[12,13] tandem mass spectrometric,[14–19] and electrochemical[10] detectors. A flow-injection chemiluminescence method has been reported for the determination of granisetron.[20]

Derivative spectrophotometry has provided an approach to overcome the problem of matrix interference in quantitative analysis. Derivative spectrophotometry has been applied for the determination of a drug in single component formulation to correct for the interference due to formulation matrix and/or to improve the spectral features of benzenoid UV-absorbing drugs. The technique has been also used for the determination of a drug in the presence of its degradation product, and analysis of binary mixtures of drugs in their pharmaceutical preparations. The applications of derivative spectrophotometry have been recently reviewed.[21]

Being an amide, GRS may undergo hydrolytic degradation reaction (Figure 1) to give 1-methyl-1H-indazole-3-carboxylic acid (I) and (1R, 3r, 5S)-9-mehtyl-9-azabicyclo[3.3.1]nonan-3-amine (II). The reaction is catalyzed by both acids and bases.

The aim of the present study is to develop a simple, first derivative spectrophotometric method for determination of granisetron in presence of its hydrolytic products, formulation adjuvant as well as the preservatives added to its pharmaceutical preparations. Validation and applications of the proposed method to pharmaceutical preparations containing granisetron are presented.

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[*] Correspondence to: Ismail I. Hewala, Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria 21521, Egypt. E-mail: hewalapda2007@hotmail.com

[a] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria 21521, Egypt

[b] Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Drug Manufacturing, Pharos University, Alexandria, Egypt

[c] Faculty of Pharmacy, University of Montreal, Montréal H3C 3J7, Canada

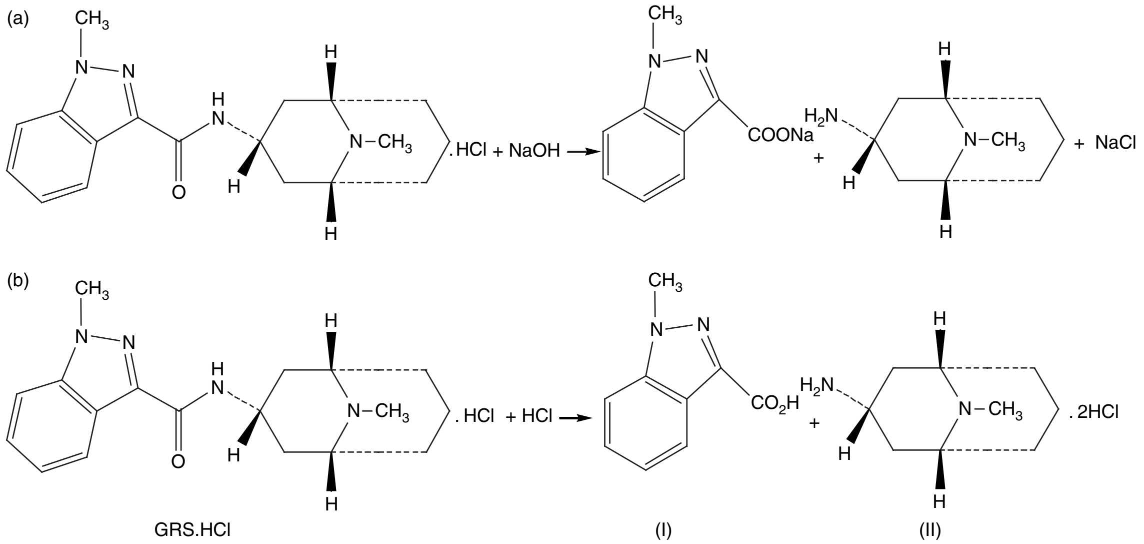

Figure 1. Schemes of (a) alkaline-induced and (b) acid-induced hydrolytic degradation of granisetron hydrochloride.

Experimental

Materials and reagents

GRS, pharmaceutical grade (Carbo Mer Inc., San Diego, CA, USA) was used and certified to contain 99.96%. Sodium hydroxide, hydrochloric acid (35%) and methanol were of analytical grade (Merck, Darmstadt, Germany). Different brands (Kytril®, Roche Pharmaceuticals, USA and Grantyl®, Alexandria Pharmaceuticals, Egypt) of GRS single-dose, preservative-free injections (labelled to contain 1 mg of GRS per ml), multi-dose vials (labelled to contain 1 mg of GRS and 10 mg of benzyl alcohol per ml) and tablets (labelled to contain 1 mg of GRS per tablet) were obtained from local market.

Instrumentation

UV-absorption measurements were carried out using a Perkin-Elmer Model Lambda EZ201 double beam UV-VIS spectrophotometer (Version 1.0). The absorption spectra were measured in 1 cm quartz cells. The spectrophotometer is connected to a Panasonic impact dot-matrix printer 24 pin KX-P 3626.

General procedure

GRS stock standard solution ($300\,\mu g\,ml^{-1}$) was prepared in methanol. GRS working standard solution ($12\,\mu g\,ml^{-1}$) was prepared in diluting solvent by dilution the appropriate volume of the stock standard solution. Diluting solvent was prepared by mixing 20 volumes of methanol and 80 volumes of 0.1 M HCl. The stock standard solution is stable for ten days when stored at 4 °C while the working solution is stable for three days when stored under the same conditions.

GRS alkaline-induced degradation products (GAD) were prepared by reflux of 30 mg of GRS with 50 ml of 4 M NaOH for 12 h. The progress of the degradation reaction was monitored by application of the HPLC method[4] to samples withdrawn every 1 h, until the disappearance of the peak of granisetron and almost no change in the area of the peaks of the two degradation products for two successive samples. The resulting solution was neutralized, transferred quantitatively into a 100-ml volumetric flask and diluted to volume with the diluting solvent. A 10-ml aliquot of the neutralized solution was transferred into a 25-ml volumetric flask and diluted to volume with the diluting solvent (GAD stock solution). Such solution contains the equivalent of completely degraded $120\,\mu g\,ml^{-1}$ of GRS i.e. $67.68\,\mu g\,ml^{-1}$ of degradation product I and $59.24\,\mu g\,ml^{-1}$ of degradation product II.

GAD working solution was prepared by diluting 4 ml of GAD stock solution to 50 ml of diluting solvent. A mixture containing GRS and GAD was prepared by diluting 2 ml of GRS stock standard solution and 4 ml of GAD stock solution to 50 ml with the diluting solvent.

The first derivative spectra of the UV-absorption spectra of the standard and a mixture containing GRS and GAD were recorded and the ^1D response at 290 nm was computed.

Assay of pharmaceutical preparations

GRS tablets test solution was prepared by weighing a quantity of the powdered tablets equivalent to 1.2 mg of the drug into a 100-ml volumetric flask using 50 ml of the diluting solvent. The contents of the flask were mixed well, sonicated for 5 min, diluted to volume and filtered. The filtrate is the tablets test solution.

GRS ampoules test solution was prepared by transferring a 1-ml aliquot of the ampoules solution into a 100-ml volumetric flask and diluting to volume with the diluting solvent.

The first derivative spectra of the UV-absorption spectra of the standard and test solutions were recorded and the ^1D response at 290 nm was computed. The percentage of intact GRS in the tablets and ampoules were calculated.

Results and discussion

Development of the method

GRS is an amide that is susceptible to hydrolytic degradation (Figure 1) reaction that is catalyzed by both acid and base resulting into carboxylic acid (I) and amine (II) in 1 : 1 molar ratio. The UV-absorption spectra of GRS and GAD solutions (Figure 2) showed that direct UV-absorbance measurements could not be used for determination of GRS in presence of its degradation products due

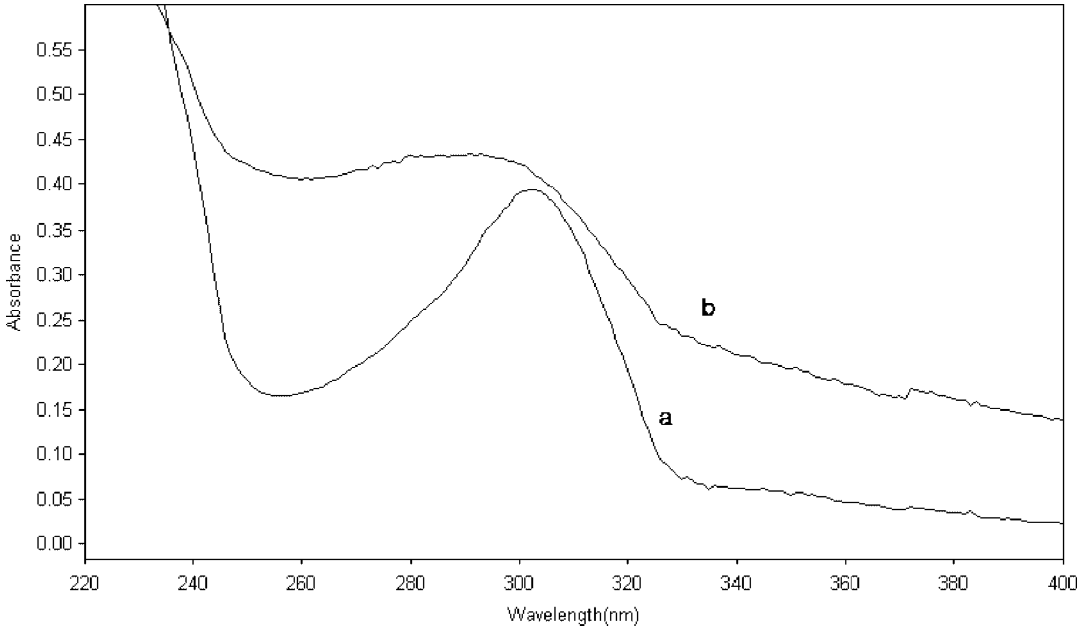

Figure 2. UV-absorption spectra of (a) standard GRS (12 µg ml^{-1}) and (b) degraded GRS (equivalent to 10 µg ml^{-1} GRS) solutions in methanol: 0.1 M HCl.

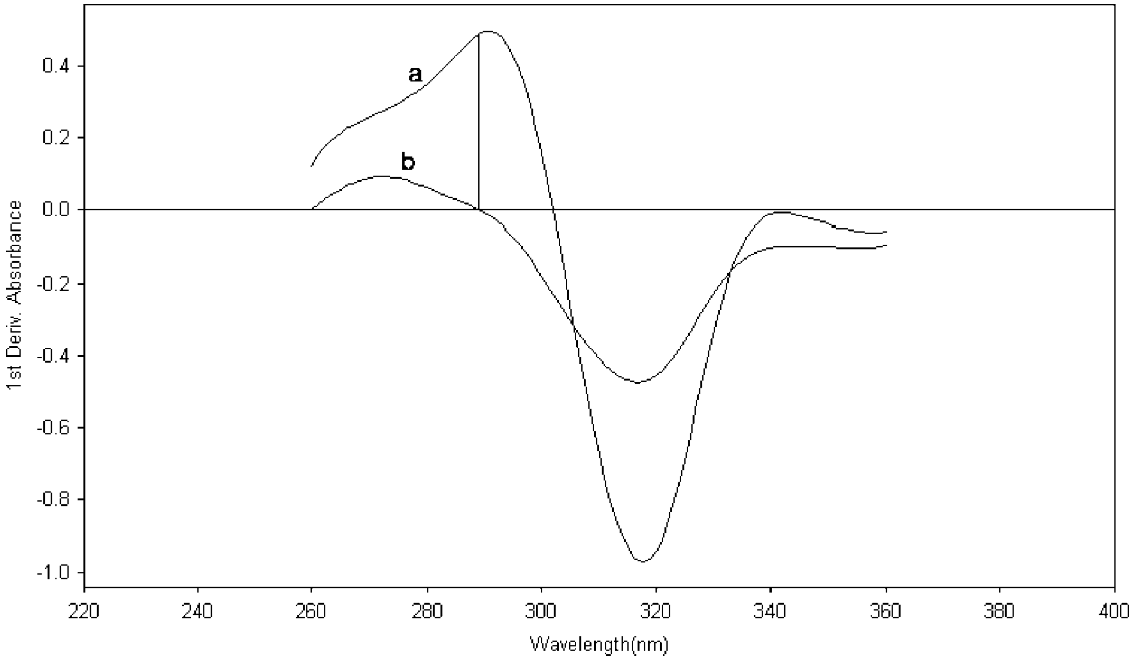

Figure 3. First derivative spectra of the UV-absorption spectra of (a) standard GRS (12 µg ml^{-1}) and (b) Degraded GRS (equivalent to 10 µg ml^{-1} GRS) solutions in methanol: 0.1 M HCl.

to the interference of the later. The first derivative spectra of the UV-absorption spectra of GRS and GAD (Figure 3) revealed that the first derivative spectra could be used for determination of GRS at the zero-crossing point of GAD at 290 nm, therefore eliminating interference from the degradation product(s). Consequently, measurement of first derivative response at 290 nm ($^1D_{290}$) for GRS solutions prepared in methanol: 0.1N HCl (20: 80) could be used for selective determination of GRS in partially degraded samples, i.e. a stability-indicating method for determination of GRS in its pharmaceutical preparations in presence of its hydrolytic products.

Validation of the proposed method

The proposed first derivative spectrophotometric method was validated according to USP analytical method validation parameters[22] and ICH guidelines.[23]

Specificity

The specificity of the proposed first derivative zero-crossing spectrophotometric method was tested by investigating the absorption and first derivative spectra of a solution containing benzyl alcohol (100 µg ml^{-1}) prepared in diluting solvent. The

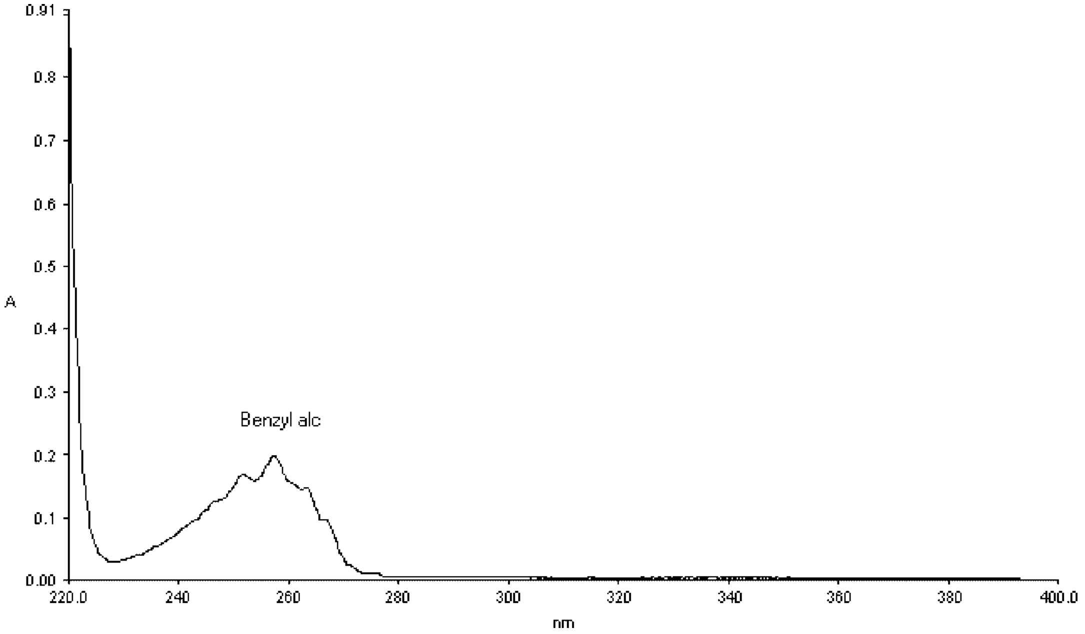

Figure 4. UV- absorption spectrum of benzyl alcohol (100 μg ml^{-1}) in methanol: 0.1 M HCl.

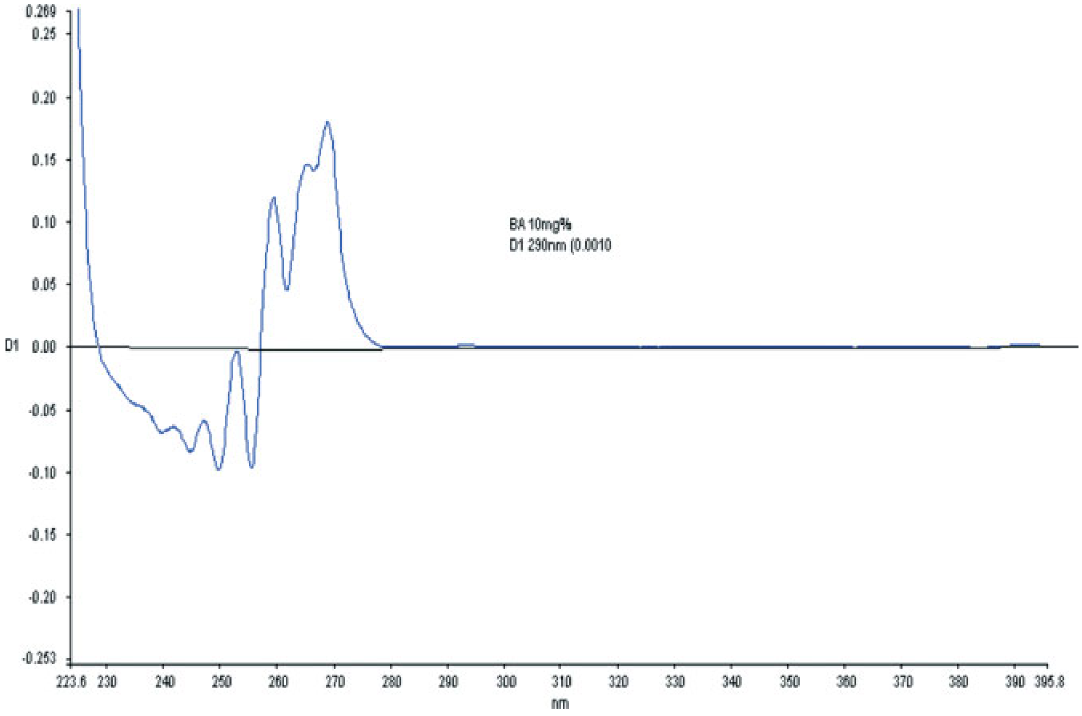

Figure 5. First derivative spectrum of the UV- absorption spectrum of Benzyl alcohol (100 μg ml^{-1}) in methanol: 0.1 M HCl.

absorption spectrum of benzyl alcohol (Figure 4) and its first derivative spectrum (Figure 5) reads zero at 290 nm. The specificity of the method was also investigated by measurement of the ^{1}D response at 290 nm ($^{1}D_{290}$) for solutions containing the co-formulated adjuvant of GRS tablets and ampoules.[3] For such solutions, $^{1}D_{290}$ read zero, i.e. do not interfere at the analytical wavelength of determination of GRS. Addition of standard GRS to such solutions resulted into $^{1}D_{290}$ readings similar to that of the corresponding concentration of GRS reference standard, which indicated that the proposed method is specific for determination of GRS in its pharmaceutical preparations without interference from its degradation products, benzyl alcohol, i.e. the preservative added to ampoules or the co-formulated adjuvant.

Linearity and range

The linearity of the proposed first derivative spectrophtpmetric method was evaluated by analyzing a series of different concentrations of GRS ranged from 50% to 150% of the analytical concentration of the method. A linear relationship existed

Table 1. Analytical performance data for the first derivative spectrophotometric determination of granisetron hydrochloride

Parameter	Value
Analytical wavelength (nm)	290
Linearity range (µg ml^{-1})	8–16
Limit of detection (µg ml^{-1})	0.1356
Limit of quantification (µg ml^{-1})	0.4109
Regression equation	
Intercept (a)	0.0146
Slope (b)	0.1651
Standard deviation of intercept (S_a)	0.0075
Standard deviation of slope (S_b)	0.0006
Standard deviation of the residuals ($S_{y/x}$)	0.0038
Correlation coefficient (r)	0.9999

between $^1D_{290}$ and the concentration of GRS. The characteristic parameters for the regression equations obtained by least squares treatment of the results are presented (Table 1). Regression analysis shows good linearity as indicated by the value of correlation coefficient (r) that is close to unity.

Selectivity

The selectivity of the proposed method for the determination of GRS in the presence of its hydrolytic products was tested by analysis of a laboratory prepared mixtures containing the analytical concentration of GRS and variable concentrations of GAD in the range of 5 to 50% of the intact GRS. The results (Table 2) indicated that the proposed method is selective and accurate for the determination of GRS bulk, i.e. raw material in the presence of its hydrolytic products.

The selectivity of the proposed method for the determination of GRS in presence of the co-formulated adjuvant, benzyl alcohol preservative and the hydrolytic products of GRS was performed by determination of GRS in the tablets and ampoules to which different concentrations of GAD were added. The results (Table 3) indicated that the proposed method is selective and accurate for the determination of GRS in the presence of the co-formulated adjuvant, the preservative added to ampoules and the hydrolytic products of GRS.

Accuracy and precision

The accuracy of the proposed first derivative spectrophotometric method for the determination of granisetron in its tablets and ampoules was evaluated in triplicate using three concentration levels; 80, 100, and 120% of the analytical concentration of the method. The results were compared with those obtained using reference method.[4] Statistical analysis[24] of the results using student's *t*-test and variance ratio F-test revealed that there is no significant difference between them regarding accuracy and precision (Table 4). The percentage relative standard deviation (RSD %) and the percentage relative error (E_r %) did not exceed 2% indicating good repeatability and accuracy of the method.

The intra-day precision was evaluated through analysis of three different concentrations in triplicate on the same day. The inter-day precision study was performed by repeating the study on three consecutive days. The results (Table 5) proved good precision as the relative standard deviation values were less than 2% for the determination of granisetron in its pharmaceutical preparations.

Robustness

The robustness of the proposed first derivative spectrophotometric method was performed by testing its capacity to be unaffected by a slight change of the percentage of methanol and 0.1 M HCl in the diluting solvent (±5%) and slight variations of the molarities of 0.1 M HCl (0.1 M ±0.01). The effect of a slight change of the analytical wavelength (±1 nm) was also investigated. These variations did not have significant effect on the measured first derivative response, $^1D_{290}$. The values of the RSD% for the measured $^1D_{290}$ after the studied variations were less than 1%.

Detection and quantification limits

The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the linearity data (Table 1) using the mathematical expressions described by the USP.[22] The values of LOD and LOQ (Table 1) indicated that the proposed first derivative method is sensitive for determination of granisetron.

Application to pharmaceutical preparations

The proposed validated spectrophotometric method was applied to the determination of granisetron in three different batches

Table 2. Determination of GRS in laboratory prepared mixtures containing GAD using the proposed first derivative sspectrophotometric method

Sample composition						
	GAD products					
GRS (µg ml^{-1})	I (µg ml^{-1})	II (µg ml^{-1})	GAD (% w/w)[a]	GRS Found ± SD[b] (µg ml^{-1})	RSD (%)[c]	E_r (%)[d]
12	0.2995	0.2620	5	12.03 ± 0.07	0.58	0.25
12	0.5990	0.5240	10	11.94 ± 0.11	0.92	−0.50
12	1.1980	1.0480	20	12.10 ± 0.09	0.74	0.83
12	1.7970	1.5720	30	12.08 ± 0.06	0.50	0.67
12	2.3960	2.0960	40	11.92 ± 0.12	1.00	−0.67
12	2.9950	2.6200	50	12.13 ± 0.08	0.66	1.08

[a] % Weight of GAD relative to weight of intact GRS.
[b] Mean ± standard deviation (SD) of three determinations.
[c] % Relative standard deviation.
[d] % Relative error.

Table 3. Determination of GRS in pharmaceutical preparations spiked with GAD using the proposed first derivative spectrophotometric method

Pharmaceutical preparation	Sample composition GRS.HCl ($\mu g\ ml^{-1}$)	GAD products I ($\mu g\ ml^{-1}$)	GAD products II ($\mu g\ ml^{-1}$)	GAD (% w/w)[a]	GRS Found ± SD[b] ($\mu g\ ml^{-1}$)	RSD (%)[c]	E_r (%)[d]
Tablets[e]							
Sample I	12	0.2995	0.2620	5	11.93 ± 0.12	1.01	−0.59
Sample II	12	0.5990	0.5240	10	12.04 ± 0.03	0.25	0.33
Sample III	12	1.1980	1.0480	20	11.89 ± 0.08	0.67	−0.92
Ampoules[f]							
Sample I	12	0.2995	0.2620	5	12.04 ± 0.09	0.75	0.33
Sample II	12	0.5990	0.5240	10	12.02 ± 0.06	0.50	0.17
Sample III	12	1.1980	1.0480	20	12.03 ± 0.05	0.42	0.25

[a] % Weight of GAD relative to weight of intact GRS.
[b] Mean ± standard deviation of three determinations.
[c] % Relative standard deviation.
[d] % Relative error.
[e] Labelled to contain 1 mg of GRS base per tablet.
[f] Labelled to contain 1 mg of GRS base and 10 mg of benzyl alcohol per 1 ml of solution.

Table 4. Determination of granisetron in pharmaceutical preparations using the proposed first derivative spectrophotometric and the reference methods

Pharmaceutical preparation	Sample number	% of Method concentration	Mean % found[a] Proposed $^1D_{290}$	Mean % found[a] Reference method[4]b
Granisetron tablets	I	70	100.09	99.99
	II	80	99.98	100.32
	III	90	99.25	99.57
	IV	100	100.58	98.16
	V	110	99.66	100.32
	VI	120	100.81	99.87
	Mean		99.89	99.71
	SD		0.73	0.81
	RSD %		0.73	0.81
	t		0.43 (2.23)	
	F		1.23 (5.05)	
Granisetron ampoules	I	70	99.89	100.05
	II	80	98.10	99.78
	III	90	100.13	99.45
	IV	100	99.08	98.81
	V	110	98.54	99.27
	VI	120	100.18	100.01
	Mean		98.32	99.73
	SD		0.88	0.76
	RSD %		0.89	0.76
	T		0.86 (0.861)	
	F		1.35 (1.346)	

[a] Mean of three determinations for each sample.
[b] Reference HPLC method.[4]
The values between brackets are the tabulated t and F values at $p = 0.05$.

of its tablets and ampoules. Satisfactory results were obtained as the mean percentage found for granisetron was in good agreement with the label claimed and with those obtained using the reference HPLC method.[4] The calculated values of the Student's *t*-test and the variance ratio F-test did not exceed the theoretical ones at the 95% confidence level indicating no significance difference between the two methods regarding the % drug found and standard deviation values. The mean percentage found and the RSD % values (Table 6) indicated that the proposed validated spectrophotometric method could be adopted for routine determination of GRS in its tablets and ampoules.

Table 5. Precision data of the first derivative spectrophotometric determination of granisetron in pharmaceutical preparations

Pharmaceutical preparation	Labelled GRS (μg ml^{-1})	Within-day precision		Between-day precision	
		Mean found[a] (μg ml^{-1})	RSD %[b]	Mean found[a] (μg ml^{-1})	RSD %[b]
Tablets[c]					
Sample I	8	7.93	1.22	7.89	1.41
Sample II	12	11.89	1.09	12.09	1.38
Sample III	16	16.14	0.89	16.22	1.66
Ampoules[d]					
Sample I	8	8.02	0.77	8.05	1.04
Sample II	12	12.05	0.46	12.10	0.98
Sample III	16	15.98	0.51	16.03	0.75

[a] Mean of three determinations.
[b] Relative standard deviation.
[c] Labelled to contain 1 mg of GRS base per tablet.
[d] Labelled to contain 1 mg of GRS base and 10 mg of benzyl alcohol per 1 ml solution.

Table 6. Application of the first derivative spectrophotometric method to the determination of granisetron in pharmaceutical preparations

Pharmaceutical preparation	Mean % found ± SD[a]	RSD %[b]
Kytril® tablets		
Batch 1	99.61 ± 0.57	0.57
Batch 2	99.15 ± 0.16	0.16
Batch 3	100.59 ± 0.62	0.62
Gratyl® tablets		
Batch 1	99.59 ± 0.93	0.93
Batch 2	99.97 ± 0.95	0.95
Batch 3	99.91 ± 0.38	0.38
Kytril® ampoules		
Batch 1	100.33 ± 0.45	0.45
Batch 2	100.21 ± 0.69	0.69
Batch 3	99.89 ± 0.77	0.77
Kytril® vials		
Batch 1	100.44 ± 0.98	0.98
Batch 2	100.66 ± 0.72	0.72
Batch 3	101.06 ± 1.11	1.10
Grantyl® ampoules		
Batch 1	100.43 ± 1.23	1.22
Batch 2	98.76 ± 0.88	0.89
Batch 3	99.51 ± 1.41	0.42

[a] Mean of three determinations ± standard deviation (SD).
[b] Relative standard deviation %.
Kytril® tablets and Grantyl® tablets labelled to contain 1 mg of GRS base per tablet.
Kytril® ampoules and Grantyl® ampoules labelled to contain 1 mg of GRS base per ml.
Kytril® vials labelled to contain 1 mg of GRS base and 10 mg of benzyl alcohol per ml.

Conclusion

In this study, a direct, simple and accurate first derivative spectrophotometric method was developed for the determination of granisetron in presence of its hydrolytic products, the co-formulated adjuvant of the pharmaceutical preparations and benzyl alcohol preservative added to granisetron parenteral preparations. The proposed method could be considered as a stability-indicating as hydrolysis is the main route of degradation of granisetron. Reviewing the literature revealed that there was no reports for the use of direct or derivative spectrophtometric-based method for determination of granisetron in presence of the hydrolytic products and benzyl alcohol. The proposed method possesses the advantages of traditional spectrophotometry being simple and rapid together with other important analytical merits such as selectivity, ruggedness and robustness. Compared with chromatographic methods, the proposed method does not require sophisticated experimental set-up associated with chromatography. Moreover, the proposed method does not require the use of large amounts of organic solvent as only small amount of methanol is used in the procedure and few minutes are required for the application of the method. Consequently, the proposed method is cost-effective and environmentally friendly. The proposed method was validated and validation acceptance criteria were met for all validation parameters and that guaranteed the reliability of the method. The assay results obtained in this study strongly encourage us to apply the validated method for the routine analysis and quality control of the pharmaceutical preparations containing granisetron.

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