STABILITY INDICATING DERIVATIVE SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF VALBENAZINE TOSYLATE IN BULK AND IN FORMULATION[**]

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Valbenazine tosylate, a benzoquinolizidine derivative, is a highly selective vesicular monoamine transporter 2 (VMAT2) inhibitor. It is the first and the only drug approved by the FDA for the treatment of tardive dyskinesia. Rapid, sensitive, and cost-effective first-order derivative UV spectrophotometric methods have been developed for the estimation of valbenazine in bulk and in its marketed formulation. Preliminary spectrophotometric determination of the drug was carried out in acetonitrile and in 0.1 N HCl with a total of 19 parametric variations for the two methods. The selected three-method variants employing peak–zero (P–0) and peak–peak (P–P) techniques were assessed for their stability, indicating potential in force degraded solutions of the drug. The developed methods were validated with respect to linearity, accuracy, precision, and robustness. Linearity was observed within the concentration range 5.0–70.0 μg/mL with an excellent correlation coefficient (r^2) of 0.9998. The limits of assay detection values for the proposed method variants were found to range from 0.89 to 2.75 μg/mL, and quantitation limits ranged from 1.19 to 4.32 μg/mL. The proposed methods were applied for the determination of the drug in its marketed capsule formulation, and percentage recovery was found to range from 94 to 95%.

Keywords: *valbenazine tosylate, spectrophotometric method, correlation coefficient.*

МЕТОДЫ ПРОИЗВОДНОЙ СПЕКТРОФОТОМЕТРИИ ДЛЯ ОПРЕДЕЛЕНИЯ ТОЗИЛАТА ВАЛБЕНАЗИНА В НЕРАСФАСОВАННОМ ВИДЕ И ФАРМАЦЕВТИЧЕСКИХ СОСТАВАХ

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Валбеназина тозилат, производное бензохинолизидина, — высокоселективный ингибитор везикулярного переноса моноаминов 2 (VMAT2), первый и единственный препарат, одобренный FDA для лечения поздней дискинезии. Для оценки валбеназина в нерасфасованном виде и фармацевтических составах разработаны чувствительные и экономически эффективные УФ-спектрофотометрические методы производной первого порядка. Предварительное спектрофотометрическое определение препарата проводили в ацетонитриле и в 0.1 N HCl с 19 вариациями параметров для двух методов. Выполненная оценка стабильности выбранных вариантов методов, в том числе "пик–ноль" (P–0) и "пик–пик" (P–P), указывает на их потенциал для растворов, подвергшихся принудительному разложению. Разработанные методы проверены на линейность, точность, прецизионность, надежность. Линейность в диапазоне концентраций 5.0–70.0 мкг/мл с коэффициентом корреляции r^2 = 0.9998. Пределы обнаружения для предложенных вариантов метода 0.89—2.75 мкг/мл, пределы

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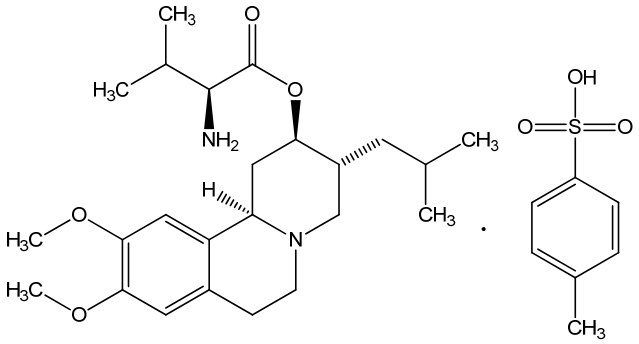
количественного определения 1.19—4.32 мкг/мл. Методы применены для оценки лекарственного средства в форме капсул, поступающих на рынок, процент извлечения от 94 до 95%.

Ключевые слова: *тозилат валбеназина, спектрофотометрический метод, коэффициент корреляции.*

Introduction. Valbenazine tosylate is chemically [(2*R*,3*R*,11bR)-9,10-dimethoxy-3-(2-methylpropyl)-2,3,4,6,7,11b-hexahydro-1*H*-benzo[a]quinolizin-2-yl](2*S*)-2-amino-3-methylbutanoate;4-methylbenzenesulfonic acid [1].



This drug is an atypical type inhibitor of the protein, vesicular monoamine transporter 2 (VMAT2). It was the first drug approved by the FDA (in 2017) for the management of tardive dyskinesia in adult patients [2, 3], where it reduces the availability of monoamine neurotransmitters by preventing their storage in synaptic vesicles [4]. Tardive dyskinesia (TD) is an abnormal hyperkinetic involuntary movement disorder [5] characterized by involuntary movements of the face, lips, tongue, trunk, and extremities [6]. It has also been reported as a serious adverse effect of antipsychotic drug medications, especially with conventional (typical) antipsychotics. A survey of the available literature reports retrieved very little information on the analytical method development for this drug. Further, there is no official pharmacopeial method for the determination of the drug. Most of the available literature reports involved the application of chromatographic techniques for the estimation of valbenazine in bulk and in pharmaceutical dosage forms [7]. To date, there has been only one stability-indicating method based on liquid chromatography, which has been reported for the estimation of valbenazine, coupled with a degradation kinetic study [8]. Recently, high-performance liquid chromatography (HPLC) study, coupled with Orbitrap mass spectrometry was reported for analysis of an analog of VAL, P109, in human liver microsomes, in the presence of its eight metabolites. The method was further used to carry out the mass spectrometric identification of the metabolites [9].

To date, there has been to our knowledge no report on the development and validation of any zero-order or higher-order UV-Visible spectrophotometric method for this drug. Derivative spectrophotometry is a versatile technique that offers several advantages over simple zero-order spectrophotometry in terms of increased sensitivity and selectivity. Hence, this highly sensitive, economical, and simple technique can be particularly useful for the development of analytical methods for the estimation of drugs present in extremely low amounts. This is the first-ever report on the development of simple, rapid, and reproducible first-order derivative spectrophotometric methods for the quantification of valbenazine tosylate in bulk and in its marketed capsule formulation. The developed methods were validated with respect to various parameters outlined in the ICH guideline Q2 (R1) [10].

Experimental. Valbenazine tosylate (Batch number 546268) was graciously provided as a gift sample by Cipla Limited, Mumbai (India). Analytical reagent (AR) grade chemicals and materials were purchased from Merck India Pvt. Ltd. (Mumbai, India) and were employed throughout the study. All solutions were freshly prepared using triple-distilled water obtained from Milli-Q plus purification system (Millipore, Billerica, MA, USA). Valbenazine capsules (label claim 40 mg per capsule INGREZZA™; Neurocrine Biosciences, San Diego, CA, USA) were purchased from the local market. All the glassware, including volumetric flasks, beakers, pipettes, measuring cylinders, and round bottomed flasks were Class A apparatus from Borosil (Mumbai, India).

All absorption spectra were recorded using a Perkin Elmer lambda 3200 UV-Visible spectrophotometer (Serial no:1906001) with a scanning speed of 60 nm/min, a spectral slit width of 2.0 nm, resolution of 2.0 nm, and equipped with 10-mm matched quartz cells. Melting point apparatus (model T0603160; EIE Instruments Pvt. Ltd., Ahmedabad, India) was used for the determination of the melting point of valbenazine.

Standard stock solution (1000 μg/mL) of valbenazine tosylate in acetonitrile or 0.1 N HCl was prepared every day, and this was diluted (1 in 10) to obtain the stock solution (100 μg/mL). Serial dilutions of the stock solution were carried out with appropriate solvents (Method A or Method B) to obtain the working standard solutions (5.0 to 70.0 μg/mL). Zero-order and first-order derivative spectra were recorded over the wavelength range of 210–400 nm against a reagent blank, and absorbance values (zero-order spectra) or amplitudes of the maximum and minimum (first-order spectra) were measured.

The drug concentration selected for stress studies was 1.0 mg/mL.

Hydrolytic studies were carried out under acidic and basic conditions by refluxing the drug in 0.1 N HCl and 0.1 N NaOH respectively at 80°C for 8 h.

Oxidative studies were carried out at room temperature in 30% hydrogen peroxide (H_2O_2) for half an hour. Thermal degradation was carried out by exposing the drug (200 mg) in a petri-dish, sealed with aluminum foil (to avoid photo-degradation), to a temperature of 60°C for 21 days. Subsequently, the petri-dish was removed, cooled to room temperature, and its contents dissolved in acetonitrile (diluent).

Two spectrophotometric methods A and B (in acetonitrile and 0.1 N HCl respectively) were explored with a total of 19 parametric variations. Amongst these, one optimized variant of method A, and two optimized variants of method B were selected and validated with respect to various parameters outlined in the ICH guideline Q2 (R1).

The working standard solution (100.0 μg/mL) was serially diluted with an appropriate reagent (acetonitrile or 0.1 N HCl) to prepare solutions with concentrations ranging from 5 to 70.0 μg/mL of the drug. All these dilutions, along with the working standard solution, prepared in triplicate, were analyzed by various zero-order and first-order spectrophotometric methods.

The intraday precision of the methods (selected based on linearity studies) was determined by the analysis of three varying concentrations of the drug (10.0, 20.0, and 30.0 μg/mL) on a single day. Determination of interday precision was carried out by analyzing three samples of varying concentrations on three successive days. The precision was expressed as RSD% corresponding to each calculated concentration of the analyte.

A pre-neutralized, equal-volume mixture of stress-degraded solutions of valbenazine tosylate prepared under conditions of acidic/alkaline hydrolysis, acid photolysis, and oxidative stress, was prepared. The original drug concentration in all the stressed solutions was the same, i.e., 10.0 μg/mL. This pre-analyzed degraded drug solution mixture of valbenazine tosylate was suitably diluted to obtain the un-spiked solution of the drug (10.0 μg/mL) for accuracy analysis. This solution was then spiked by 50, 100, and 150% to provide concentration increases by 5.0, 10.0, and 15.0 μg/mL respectively by mixing the unfortified solution separately with equal volumes of the standard drug solutions of strengths of 20.0, 30.0, and 40.0 μg/mL respectively. The drug concentration in the fortified solutions (final analyzed concentrations of 15.0, 20.0, and 25.0 μg/mL) and the unfortified solution, were then determined (n = 3). Method accuracy was expressed as the percentage recovery of the fortified drug concentration with reference to the unfortified one.

Robustness was assessed by carrying out deliberate changes in the method variables, including temperature and pH, and studying their impact on the recovery of the drug in the test solutions.

Twenty capsules of valbenazine tosylate (INGREZZA™, Neurocrine Biosciences) with a label claim of 40 mg per capsule, were emptied and the mixed powder weight equivalent to 10.0 mg of valbenazine was dissolved in an appropriate reagent (acetonitrile or 0.1 N HCl), to prepare 100 mL of the solution A (1000 μg/mL). The solution was suitably diluted and analyzed for the drug content by three method variants developed.

Results and discussion. Derivative spectrophotometry offers significant advantages over zero-order spectrophotometry in terms of enhanced specificity and selectivity. The selection of appropriate peak amplitudes in the derivative curves can permit drug analysis in the presence of excipients, degradation products, and other impurities. In this light, a comprehensive study was carried out to thoroughly explore all possible zero- and first-order derivative spectrophotometric curves of valbenazine tosylate to develop sensitive and reproducible stability-indicating methods for the drug.

A preliminary analysis of UV absorption and solubility characteristics of the drug was carried out to select an appropriate solvent system for method development. The computed log P of valbenazine tosylate is 3.65 and the pK_a of the drug (strongly basic) is 8.41 [1]. The drug has slight solubility in water (0.0383 mg/mL). The drug demonstrates best solubility characteristics in acidic medium, although good solubility is seen in acidic, basic, as well as neutral aqueous media. Considering the solubility profile of valbenazine tosylate, acetonitrile and 0.1 N HCl were selected for the UV spectrophotometric method development and validation. The zero- and first-order derivative spectra for the standard solutions of valbenazine

tosylate, ranging from 5.0 to 70.0 μg/mL, were recorded over the wavelength range 210–400 nm, taking acetonitrile or 0.1 N HCl as the corresponding reagent blank. The amplitudes of the maxima and minima were measured for all derivative spectra. Figure 1 shows the zero-order and first-order derivative UV overlay spectra of valbenazine tosylate in acetonitrile and 0.1 N HCl.

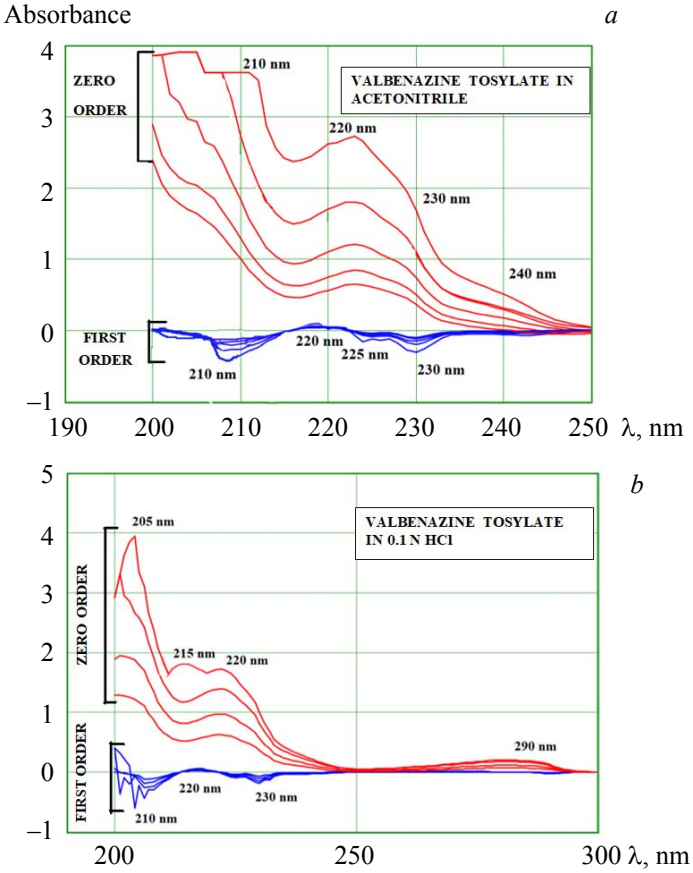


Fig. 1. Zero-order and first-order derivative UV overlay spectra of valbenazine tosylate (a) in acetonitrile and (b) in 0.1 N HCl.

The regression parameters, Beer's law limits, and wavelength range for the working standard solutions of valbenazine tosylate employing 19 variants of methods A and B (zero-order and first-order) are summarized in Table 1. Four zero-order and four first-order derivative UV spectrophotometric variants were studied for method A (in acetonitrile), whereas four zero-order and seven first-order derivative UV spectrophotometric variants were studied for method B (in 0.1 N HCl). Amongst these, one variant of method A (12), and two variants of method B (15 and 18) were selected for further validation, as peak amplitudes (zero or peak-to-peak) afforded the best linear correlation in these methods. Figure 2 shows the standard plots obtained for the analysis of valbenazine tosylate with the selected method variants.

The method was validated with respect to linearity and range, accuracy and precision, the limit of detection (LOD), and the limit of quantification (LOQ). The various method validation parameters are summarized in Tables 1 and 2.

The absorbance measurements (in the case of zero-order spectra) and the peak-to-zero (P–0) or peak-to-peak (P–P) amplitude measurements (in the case of first-order derivative spectra) were done at varying wavelengths within the concentration range 5.0–70.0 μg/mL of the drug. The various regression parameters corresponding to the different variants of methods A and B are summarized in Table 1. Values of the correlation coefficient r^2 were found to be above 0.9 in many cases, indicating good linearity over the working concentration ranges. Method variants returning the best r^2 values (close to 1.0), were selected for further analytical validation, (i.e., methods 12, 15, and 18).

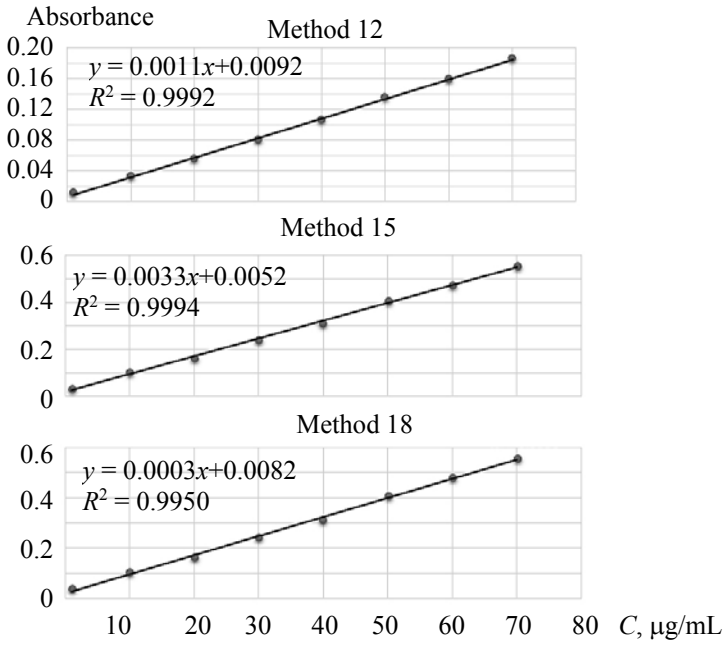


Fig. 2. Standard plots of valbenazine tosylate with method variants 12, 15, and 18.

TABLE 1. Linearity and Range for the Explored Methods for the Analysis of Valbenazine Tosylate by Zero-order and First-order Derivative Spectrophotometry

Method No.	Method type	Method variant	Beer's law limits, μg/mL	λ, nm	Technique	Regression equation	r^2
1	A	Zero order	5–70	230	Abs	$y = 0.0012x - 0.0021$	0.9995
2	A	Zero order	5–50	210	Abs	$y = 0.0021x - 0.0034$	0.9994
3	A	Zero order	5–50	220	Abs	$y = 0.0027x - 0.0009$	0.9978
4	A	Zero order	5–70	240	Abs	$y = 0.0025x - 0.0064$	0.9982
5	B	Zero order	20–50	205	Abs	$y = 0.0049x - 0.0048$	0.9955
6	B	Zero order	20–50	215	Abs	$y = 0.0040x + 0.0450$	0.9967
7	B	Zero order	20–50	220	Abs	$y = 0.0058x - 0.0023$	0.9960
8	B	Zero order	20–80	290	Abs	$y = 0.0025x + 0.0078$	0.9967
9	A	First order	5–50	210	P–0	$y = 0.0019x + 0.0058$	0.9989
10	A	First order	5–30	220	P–0	$y = 0.0010x + 0.0091$	0.9983
11	A	First order	5–30	225	P–0	$y = 0.0015x + 0.0035$	0.9987
12	A	First order	5–50	230	P–0	$y = 0.0011x + 0.0092$	0.9952
13	B	First order	20–50	210	P–0	$y = 0.0042x - 0.0087$	0.9991
14	B	First order	20–50	220	P–0	$y = 0.0029x + 0.0052$	0.9968
15	B	First order	20–50	230	P–0	$y = 0.0033x - 0.0052$	0.9994
16	B	First order	5–30	207–210	P–P	$y = 0.0028x - 0.0096$	0.9976
17	B	First order	20–80	228–230	P–P	$y = 0.0032x - 0.0018$	0.9912
18	B	First order	20–50	205–210	P–P	$y = 0.0003x - 0.0082$	0.9990
19	B	First order	20–50	217–220	P–P	$y = 0.0016x - 0.0014$	0.9982

N o t e. A = Calibration data in acetonitrile. B = Calibration data in 0.1 N HCl.

The LOD and LOQ of the method were established using calibration standards. LOD and LOQ were calculated as $3.3\sigma/s$ and $10\sigma/s$, respectively, as per ICH definitions, where σ is the mean standard deviation of replicate determination carried out under the same conditions as the sample analysis in the absence of the analyte (blank determination), and s is the sensitivity, namely, the slope of the calibration graphs. LOD and LOQ values for all method variants 12, 15, and 18 were found to be 1.14, 0.89, and 2.75 μg/mL, and 2.43, 1.19, and 4.32 μg/mL, respectively.

TABLE 2. Validation Parameters for the Proposed Method Variants

Parameter	Valbenazine tosylate					
Accuracy	Concentration (μg/mL) ± S.D., %RSD[c]					
Conc. of drug taken, μg/mL	Conc. of std added, μg/mL[a]	Calculated[b], % Recovery by method variants				
		12		15	18	
10.0	5.0 (50%)	91.28±0.01, 2.17		97.16±0.01, 0.27	94.15±0.01, 1.15	
10.0	10.0 (100%)	95.43±0.01, 1.44		92.67±0.01, 0.31	95.07±0.01, 1.09	
10.0	15.0 (150%)	93.56±0.01, 0.65		98.22±0.01, 0.22	97.44±0.01, 1.18	
Precision	Calculated conc. ± S.D.; %RSD[c] with method variants 12, 15, and 18					
Conc. taken, μg/mL	Intra-day (n = 3)			Inter-day (n = 3)		
	12	15	18	12	15	18
10.0	9.11±0.01, 2.55	9.69±0.01, 0.54	9.22±0.01, 2.10	9.24±0.01, 0.12	9.19±0.01, 2.29	9.14±0.01, 1.26
20.0	19.55±0.01, 0.60	19.22±0.06, 1.88	19.38±0.01, 0.43	19.23±0.01, 0.39	19.45±0.01, 0.98	19.61±0.01, 0.25
30.0	27.45±0.01, 2.40	28.05±0.01, 0.66	28.33±0.01, 2.17	29.87±0.01, 1.26	29.23±0.01, 1.15	28.55±0.01, 0.13
LOD, μg/mL	1.14 (method 12), 0.89 (method 15), 2.75 (method 18)					
LOQ, μg/mL	2.43 (method 12), 1.19 (method 15), 4.32 (method 18)					

[a] Equal volumes of standard drug solutions (20, 30, and 40 μg/mL) added to pre-analyzed drug solution (10 μg/mL).
[b] Calculated as a mean of measurements in triplicate (n = 3).
[c] Calculated as: SD/mean ×100.

Precision was investigated by analyzing three different concentrations of valbenazine tosylate (10.0, 20.0, and 30.0 μg/mL) in three independent repeats on the same day (to evaluate intraday precision) and on three consecutive days (to evaluate inter-day precision). These intraday and inter-day precision data, represented as relative standard deviation (RSD%), are shown in Table 2. The RSD% values in the intraday and the inter-day precision study were found to be less than 2.55 and 2.29% respectively, for method variants 12, 15, and 18 indicating good precision of the method.

The stability indicating the potential of the developed methods was evaluated by fortifying a pre-neutralized, equal-volume mixture of stress-degraded solutions of valbenazine tosylate prepared under conditions of acidic/alkaline hydrolysis, acid photolysis, and oxidative stress. The original drug concentration in all the stressed solutions was the same, i.e., 10.0 μg/mL. The accuracy of the proposed methods was assessed by preparing different concentration levels of the drug for analysis from independent stock solutions. Further assessment of the accuracy of the developed methods was carried out by spiking excess drug (50, 100, and 150%) to pre-analyzed degraded drug solution samples (10.0 μg/mL). Accuracy was determined as mean % recovery and RSD%. Excellent recovery values for method variants 12, 15, and 18 ranging from 91.28–98.22% (Table 2) indicated good accuracy of the method.

Robustness gives the measure of the repeatability of an analytical method, which is assessed by evaluating the effect of small variances in experimental conditions such as heating temperatures (± 2°C). Three replicate determinations at six different concentration levels of the drugs were carried out at ambient temperature (26°C) and at 28 and 23°C (room temperature ±2°C). The intraday %RSD values for the method variants 12, 15, and 18 were found to be less than 0.43%, indicating that the proposed method variants have reasonable robustness.

The stability of the final sample solutions was examined by their absorbance values/peak amplitudes, and responses were found to be stable for at least 6 h at room temperature.

Table 3 shows the results of the assay for valbenazine tosylate carried out on the marketed formulation by the proposed three method variants. The percentage recovery was found to range from 94–95% showing a close agreement between the results obtained by the proposed method variants and the label claim.

TABLE 3. Recovery Studies from Marketed Drug Formulation INGREZZA™ capsules, Neurocrine Biosciences

Method No.	Label claim, mg	Mean recovery* (mg)±S.D, %RSD	% Recovery ± S.D, %RSD
1	40	38.05±2.19, 1.05	95.12±0.07, 1.18
2	40	37.69±1.70, 1.28	94.22±0.01, 2.05
3	40	38.13±2.27, 1.55	95.32±0.03, 2.65

* Average of three determinations.

Conclusions. Three variants of a rapid, sensitive, inexpensive, and accurate zero-/first-derivative method have been developed for quantification of valbenazine tosylate in bulk, as well as in its marketed formulation (capsules). The method variants were validated in terms of their sensitivity, reproducibility, precision, accuracy, robustness, and solution stability for ≥6 h, suggesting their suitability for routine analysis of the drug in pure form (bulk analysis) as well as in pharmaceutical formulations, without interference from excipients. To our knowledge, this is the first ever report on the development of a UV spectrophotometric method for valbenazine tosylate as a stability-indicating method. Excellent recovery of the drug from its force-degraded solutions suggests the stability-indicating nature of the method and its potential applicability in the presence of routine degradation products. We have comprehensively explored all wavelength regions in the zero-order and first-order derivative spectra of valbenazine tosylate for its estimation (method variants 1–19). The validation parameters were found to be the best for method variants 12, 15, and 18. The proposed methods have been successfully used to quantify the drug in its marketed capsule formulation with good recoveries, suggesting that the method might be well suited for routine drug analysis without any interference from the formulation excipients. These methods can be explored further for analysis of valbenazine in other formulations containing varied excipients.

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