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Research Article

Development and Validation of RP-HPLC Method for Quantitative Estimation of Vinpocetine in Pure and Pharmaceutical Dosage Forms

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A simple, precise, specific, and accurate reversed phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for determination of vinpocetine in pure and pharmaceutical dosage forms. The different analytical performance parameters such as linearity, accuracy, specificity, precision, and sensitivity (limit of detection and limit of quantitation) were determined according to International Conference on Harmonization ICH Q2 (R1) guidelines. RP-HPLC was conducted on Zorbax C_{18} (150 mm length × 4.6 mm ID, 5 μm) column. The mobile phase was consisting of buffer (containing 1.54% w/v ammonium acetate solution) and acetonitrile in the ratio (40 : 60, v/v), and the flow rate was maintained at 1.0 mLmin^{-1}. Vinpocetine was monitored using Agilent 1200 series equipped with photo diode array detector (λ = 280 nm). Linearity was observed in concentration range of 160–240 μgmL^{-1}, and correlation coefficient was found excellent (R^2 = 0.999). All the system suitability parameters were found within the range. The proposed method is rapid, cost-effective and can be used as a quality-control tool for routine quantitative analysis of vinpocetine in pure and pharmaceutical dosage forms.

1. Introduction

Vinpocetine is a synthetic ethyl ester of apovincamine, a vinca alkaloid obtained from the leaves of the Lesser Periwinkle (Vinca minor) and discovered in the late 1960s [1]. It is a novel vasodilating agent widely used to treat acute and chronic stroke [2–5]. It also has a potential role in the treatment of Parkinson's disease and Alzheimer's disease [6, 7]. Vinpocetine selectively inhibits voltage-sensitive Na^+ channels, resulting in a dose-dependent decrease in evoked extracellular Ca^{++} ions in striatal nerve endings [8] and also inhibits IKK preventing IκB degradation and the following translocation of NF-κB to the cell nucleus [6, 7]. Chemically, it is 14-ethoxycarbonyl-(3α, 16α-ethyl-14, 15-eburnamenine) [9], as shown in Figure 1, with molecular formula of $C_{22}H_{26}N_2O_2$ and molecular weight of 350.454.

From the literature survey, it was found that various methods were used for the estimation of vinpocetine such as spectrophotometric method, high performance liquid chromatographic (HPLC) method [10], and gas chromatographic-mass spectrometric (GC-MS) method [11] in laboratory-prepared mixture, pharmaceutical preparation, and biological matrices such as human plasma. However, the aim of the present work is to develop a simple, precise, specific, accurate, cost-effective, and validated RP-HPLC method according to USP and ICH guidelines [12, 13]

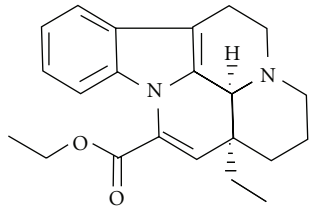


Figure 1: Chemical structure of vinpocetine.

for the estimation and routine analysis of vinpocetine in pure and pharmaceutical formulations.

2. Experimental

2.1. Reagents and Materials. Vinpocetine tablets were prepared from ACI Ltd., Narayanganj-1400, Bangladesh. Each tablet contained vinpocetine (5 mg, based on 100% potency) as an active ingredient, and microcrystalline cellulose (Avicel PH 102), sodium starch glycolate (SSG), colloidal silicon dioxide (Aerosil-200), and magnesium stearate as excipients. HPLC grade acetonitrile and reagent grade ammonium acetate were used for analytical purposes. Milli-Q water was used to prepare the mobile phase.

2.2. Preparation and Characterization of Vinpocetine Tablets (Avintol 5 mg Tablet)

2.2.1. Tablets Preparation. The immediate release vinpocetine tablet was prepared by direct compression method. The required amount of the active drug, Avicel PH 102 (diluent), and SSG (super disintegrant) was mixed by geometrical order and then sieved the granules through no. 24 sized mesh. Finally the granules were mixed with Aerosil-200 (glidant) and magnesium stearate (lubricant) passing through no. 40 sized mesh. Then the granules were compressed in single rotary Mini compressed machine using "D"-Tooling of punch size 6.0 mm in diameter (round shape), 8 station. The tablets were then film coated with ethyl cellulose (EC) and hydroxypropyl methylcellulose (HPMC) mixed with the plasticizer polyethylene glycol 6000 and titanium dioxide as opacifier.

2.2.2. Tablets Characterization. The tablets obtained were round and convex shaped with a break line on the upper side and the lower side was plain. The average weight of the tablets varied from 90 mg ± 7.5% [14]. The disintegration test of both core and coated tablets [15] and the friability test of core tablets [16] were found well within the British Pharmacopoeia (BP) acceptable limit.

2.3. Chromatographic Conditions

2.3.1. Chromatographic Parameters. An Agilent HPLC model integrated with variable wavelength programmable photo diode array detector was employed for the investigation. The chromatographic analysis was performed on a Zorbax C_{18}, 150 mm length × 4.6 mm ID with 5 μm particle size column. The mobile phase was buffer (containing 1.54% w/v ammonium acetate solution): acetonitrile (40 : 60, v/v) pumped at a flow rate of 1.0 mLmin^{-1}. The column temperature was maintained at 30°C, and the detection wavelength was 280 nm. The injection volume was 10 μL, and the run time was 15 min for each injection. HPLC grade acetonitrile was used as diluent during the standard and test sample preparation.

2.3.2. Preparation of Mobile Phase. 6.16 gm of ammonium acetate was weighted and dissolved in 400 mL of distilled water. This solution was mixed with 600 mL of HPLC grade acetonitrile and mixed well. The resulting solution was sonicated for 5 min using ultrasonic bath, and finally this solution was filtered using 0.2 μm filter.

2.3.3. Preparation of Stock Solution of Standard Vinpocetine. The stock solution of vinpocetine was prepared by dissolving 200 mg of standard vinpocetine to 100 mL with acetonitrile to give a concentration of 2 mgmL^{-1}. The solution was sonicated for 5 min using ultrasonic bath and then filtered through 0.2 μm disk filter.

2.3.4. Preparation of Assay Sample Solution. For the analysis of the tablet dosage form, not less than 20 tablets were crushed and then powdered finely. To prepare assay sample solution, powdered sample equivalent to 10 mg of vinpocetine was weighed and transferred to a clean and dry 50 mL volumetric flask. About 30 mL of acetonitrile was added as diluting solution and shaken thoroughly to extract the drug from the excipients and then sonicated for 5 min for complete dissolution of drug. The solution was allowed to cool at room temperature and then the volume was made upto the mark with the same diluting solution. The solution was filtered through Whatman filter paper (No. 42) and then finally filtered through 0.2 μm disk filter. The drug concentration of the resulting sample solution was determined by HPLC using the calibration curve of standard solution. All determinations were conducted in triplicate. To validate the proposed method, the different analytical performance parameters such as linearity, accuracy, specificity, precision, sensitivity (limit of detection and limit of quantitation), and system suitability were determined according to ICH guidelines [13].

2.4. Analytical Method Validation Parameters

2.4.1. System Suitability. To assess system suitability of the method, the repeatability, theoretical plates, tailing factor and retention time of six replicate injections of standard vinpocetine of concentration 200 μgmL^{-1} were used and the %RSD values were calculated in each case.

2.4.2. Linearity. The linearity was analyzed through the standard curves ranging from 160 to 240 μgmL^{-1} by diluting appropriate amounts of vinpocetine stock solution (2000 μgmL^{-1}) with acetonitrile and prepared in triplicate.

Three calibration curves were prepared in the same day with the following concentrations (160, 180, 200, 220, and 240 μgmL^{-1}). The linearity was evaluated by linear regression analysis, which was calculated by the least-square regression analysis.

2.4.3. Specificity. The specificity of the developed HPLC method for the determination of vinpocetine in bulk drug and pharmaceutical preparation (Avintol 5 mg tablet) was investigated by chromatographic analysis of the following.

Noninterference of Placebo. To check the noninterference of placebo, placebo solution was prepared in the same way of the sample solution in the presence of all inactive ingredients of the Avintol 5 mg tablet formulation but without vinpocetine.

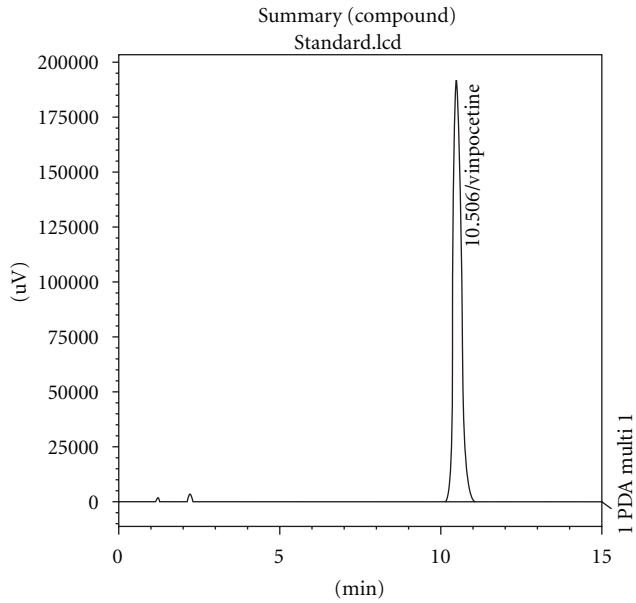
Degradation Studies. For degradation studies, 10 mg of vinpocetine was accurately weighed and transferred to a 50 mL volumetric flask. To this, 1 mL 1N HCl (for acid degradation study) or 1 mL of 1N NaOH (for alkaline degradation study) was added and kept in a stability chamber (25 ± 2°C) for 8 h. The mixture was dissolved and made to volume with acetonitrile. For photolytic degradation, nominal standard solution of vinpocetine (200 μgmL^{-1}) was exposed to UV light at 254 nm for 1 h. The final solution was injected for analysis and the presence of interfering peak(s) eluted at/or near the retention time of vinpocetine was also checked. All determinations were conducted in triplicate.

Peak Purity Evaluation. The peak purity tool was used to check the peak purity of the test solution.

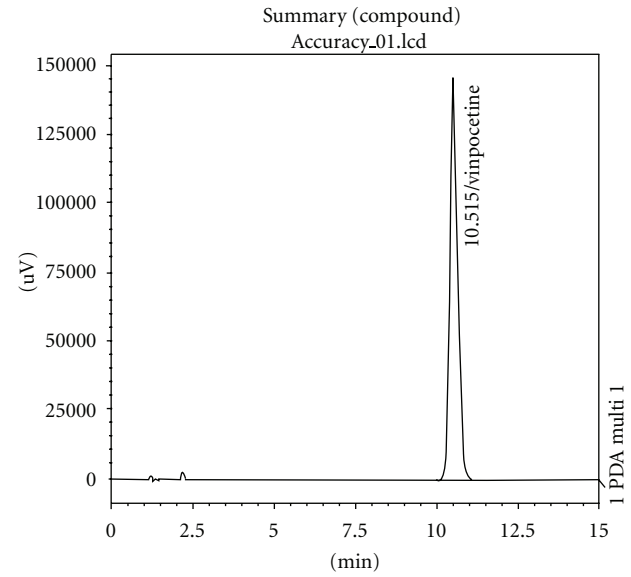
2.4.4. Accuracy. Accuracy study of the method was carried out for both drug and drug-matrix solutions. In case of drug solution, standard solutions of vinpocetine, corresponding to 80, 90, 100, 110, and 120% of the nominal analytical concentration of vinpocetine (200 μgmL^{-1}) were compared with reference standard solution of vinpocetine of known purity (200 μgmL^{-1}), and the percent recoveries (mean ± %RSD of three replicates) of vinpocetine in pure form were calculated. In case of drug-matrix solution, accuracy parameter was determined by the recovery test, which consisted of adding known amounts of vinpocetine to the samples' solutions in the beginning of the process. This test was realized by assaying five different solutions, three replicates each, containing 160, 180, 200, 220, and 240 μgmL^{-1} of vinpocetine standard solution added to vinpocetine sample solution, corresponding to 80, 90, 100, 110, and 120% of the nominal analytical concentration of vinpocetine (200 μgmL^{-1}), and the percent recoveries (mean ± %RSD of three replicates) of vinpocetine in drug-matrix form were calculated.

2.4.5. Precision. Precision of the method was determined by repeatability (intraday precision) and intermediate precision (interday precision) of both standard and sample solutions. Precision was determined in six replicates of both vinpocetine standard solution (200 μgmL^{-1}) and sample solution (200 μgmL^{-1}) on the same day (intra-day precision) and daily for 6 times over a period of one week (inter-day precision). The results were expressed as %RSD of the measurements.

2.4.6. Sensitivity. Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined using calibration

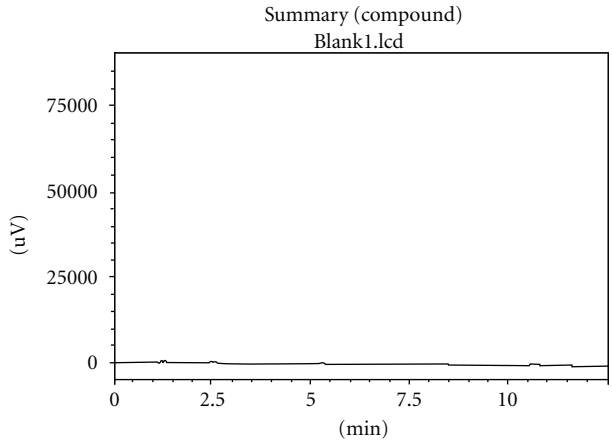


(a) Chromatogram of vinpocetine standard preparation

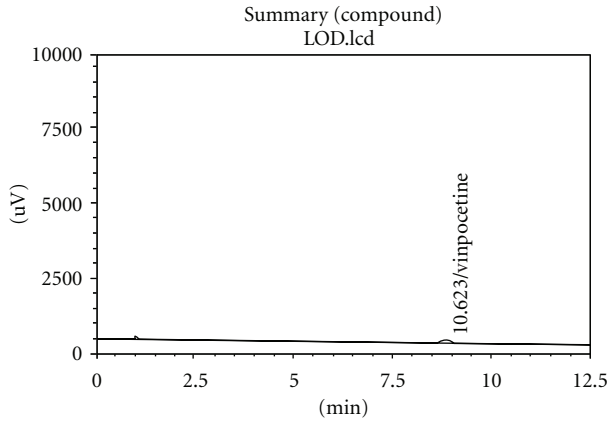


(b) Chromatogram of vinpocetine test sample

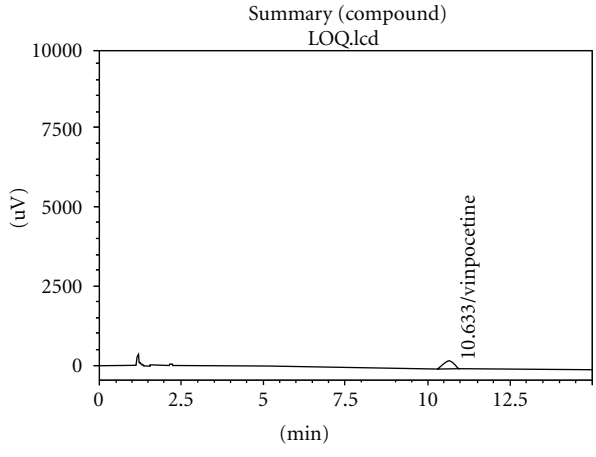
Figure 2: Typical chromatogram of (a) vinpocetine standard preparation and (b) vinpocetine test sample. (Chromatographic conditions-Zorbax C_{18}, 150 mm length × 4.6 mm ID with 5 μm particle size column; mobile phase-buffer (containing 1.54% w/v ammonium acetate solution): acetonitrile (40 : 60, v/v); flow rate 1.0 mLmin^{-1}; column temperature 30°C; wavelength 280 nm and injected volume 10 μL).



(a) Chromatogram of blank



(b) Chromatogram of LOD of vinpocetine



(c) Chromatogram of LOQ of vinpocetine

Figure 3: Typical chromatogram of (a) blank, (b) LOD of vinpocetine, and (c) LOQ of vinpocetine. (Chromatographic conditions-Zorbax C_{18}, 150 mm length × 4.6 mm ID with 5 μm particle size column; mobile phase-buffer (containing 1.54% w/v ammonium acetate solution): acetonitrile (40 : 60, v/v); flow rate 1.0 mLmin^{-1}; column temperature 30°C; wavelength 280 nm and injected volume 10 μL).

Table 1: Chromatographic characteristics of system suitability solution.

Parameter	Value (Mean ± %RSD)*
Peak area	3457550 ± 0.096
Tailing factor	1.109 ± 0.378
Theoretical plate	2945.667 ± 0.099
Retention time	10.581 ± 0.083

* Mean and % Relative Standard Deviation of six replicates.

curve method according to ICH Q2 (R1) recommendations. The LOD ($k = 3.3$) and LOQ ($k = 10$) of the proposed method were calculated using the following equation:

$$A = k\sigma/S, \tag{1}$$

where A is LOD or LOQ, σ is the standard deviation of the response, and S is the slope of the calibration curve.

2.4.7. Ruggedness. Ruggedness of the current method was determined by analyzing six assay sample solutions of Avintol 5 mg tablet formulation having concentration of 200 μgmL^{-1} by two analysts in the same laboratory to check the reproducibility of the test result. The % recovery and standard deviation were calculated in both cases.

2.4.8. Robustness. To determine the robustness of the current method, the effect of flow rate was studied at 0.9 and 1.1 mLmin^{-1} instead of 1.0 mLmin^{-1}. The effect of column temperature was studied at 25 and 35°C instead of 30°C. The effect of mobile phase composition was assessed at (Buffer : ACN = 38 : 62, v/v) and (Buffer : ACN = 42 : 58, v/v) instead of (Buffer : ACN = 40 : 60, v/v). The %RSD of robustness testing under these conditions was calculated in all cases.

3. Results and Discussion

3.1. Method Validation

3.1.1. System Suitability. The results (Mean ± %RSD of six replicates) of the chromatographic parameters are shown in Table 1, indicating the good performance of the system.

3.1.2. Linearity. The regression equation for vinpocetine was found $y = 17563x - 50470$ by plotting peak area (y) versus the concentration (x) studied from 160 to 240 μgmL^{-1}, and the correlation coefficient ($R^2 = 0.999$) was highly significant. The validity of the assay was verified by means of the ANOVA. According to it, there is linear regression and there is no deviation from linearity ($P < 0.05$).

3.1.3. Specificity. A typical HPLC chromatogram of vinpocetine standard preparation (a) and vinpocetine test sample (b) are shown in Figure 2. The HPLC chromatograms recorded for the mixture of the inactive ingredients revealed no peaks within retention time around 10.5 minutes, and the peak purity was 99.99%. Figure 2 and the peak purity index show

Table 2: Results of vinpocetine exposed to different degradative pathways.

Parameter	Amount added (μgmL^{-1})	Amount recovered (μgmL^{-1})*	Percentage of degradation
Acidic degradation	200	197.32	1.34
Alkaline degradation	200	190.86	4.57
Photolytic degradation	200	198.06	0.97

* Mean of three replicates.

Table 3: Accuracy studies of vinpocetine in standard and drug-matrix solutions.

	Amount added (μgmL^{-1})	Peak area	Amount recovered (μgmL^{-1})	% Recovery	Recovery (Mean ± %RSD)
Standard solution	160	2758830	160.12	100.08	100.05 ± 0.023
	160	2757741	160.06	100.04	
	160	2757692	160.05	100.04	
	180	3108421	180.41	100.23	100.24 ± 0.012
	180	3108988	180.44	100.25	
	180	3109134	180.45	100.25	
	200	3463527	201.02	100.51	100.52 ± 0.009
	200	3463889	201.04	100.52	
	200	3464117	201.05	100.53	
	220	3811741	221.23	100.56	100.56 ± 0.007
	220	3812077	221.25	100.57	
	220	3811562	221.22	100.56	
	240	4168397	241.93	100.80	100.79 ± 0.012
	240	4167556	241.88	100.78	
	240	4167502	241.88	100.78	
Drug-matrix solution	160	2773303	160.96	100.60	100.57 ± 0.024
	160	2772297	160.90	100.56	
	160	2772063	160.89	100.56	
	180	3119142	181.03	100.60	100.63 ± 0.030
	180	3121001	181.14	100.66	
	180	3119725	181.06	100.62	
	200	3477174	201.81	100.90	100.91 ± 0.015
	200	3477005	201.80	100.90	
	200	3477987	201.86	100.92	
	220	3831775	222.39	101.10	101.08 ± 0.025
	220	3831000	222.35	101.08	
	220	3829872	222.28	101.05	
	240	4185580	242.92	101.20	101.21 ± 0.011
	240	4186378	242.97	101.22	
	240	4185596	242.92	101.20	

Table 4: Intraday and interday precision of HPLC method.

Sr. no.	Concentration (μgmL^{-1})	Standard solution		Sample solution	
		Intra-day precision	Inter-day precision	Intra-day precision	Inter-day precision
1	200	3479501	3484566	3481332	3489214
2	200	3479005	3484112	3481987	3489741
3	200	3478987	3483991	3482244	3490301
4	200	3479227	3484977	3481510	3489748
5	200	3479808	3485004	3481993	3489849
6	200	3479777	3484669	3482214	3490488
Mean		3479384	3484553	3481880	3489890
SD		367.100	425.941	375.626	453.331
%RSD		0.011	0.012	0.011	0.013

Table 5: Ruggedness of the method.

Sample	Taken amount of standard vinpocetine (mg)	Analyst-1		Analyst-2	
		Amount found (mg)	% Recovery ± SD*	Amount found (mg)	% Recovery ± SD*
Avintol 5 mg Tablet	10	10.04	100.4 ± 0.08	10.01	100.1 ± 0.09

* % of Recovery ± Standard Deviation of six samples (assay).

Table 6: Robustness of the method.

Parameter		Amount of vinpocetine added (μgmL^{-1})	Amount of vinpocetine detected (Mean ± SD)*	%RSD
Change in mobile phase composition	Buffer : ACN = 38 : 62	200	200.33 ± 0.29	0.14
	Buffer : ACN = 40 : 60	200	200.47 ± 0.29	0.14
	Buffer : ACN = 42 : 58	200	200.56 ± 0.55	0.27
Change in column temperature	25°C	200	200.43 ± 0.71	0.35
	30°C	200	200.36 ± 0.50	0.23
	35°C	200	199.88 ± 0.56	0.28
Change in flow rate	0.9 mLmin^{-1}	200	200.08 ± 0.65	0.33
	1.0 mLmin^{-1}	200	200.41 ± 0.31	0.15
	1.1 mLmin^{-1}	200	200.46 ± 0.61	0.31

* Mean ± Standard Deviation of six replicates.

that vinpocetine is clearly separated from the response of any interfering peak(s).

Under acidic condition (1N HCl for 8 h), it was found that 1.34% of vinpocetine content was decreased, but there was no detectable degradation peak(s). The samples submitted to alkaline condition (1N NaOH for 8 h) showed 4.57% degradation of vinpocetine content, and also there was no detectable degradation peak(s). In both cases, the peak purity was 99.99%. Again, when the sample was submitted to photolytic degradation (UV light at 254 nm for 1 h), the degradation of vinpocetine was found 0.97% and the peak purity was 99.99%. The results obtained from the peak purity tool demonstrated that the peak response of vinpocetine was pure in all cases and thus confirming the absence of other substance in the same retention time. The results of percentage of forced degradations are shown in Table 2.

3.1.4. Accuracy. The results were expressed as percent recoveries of the particular components in the samples. The overall results of percent recoveries (mean ± %RSD) of vinpocetine in pure and drug-matrix solutions are demonstrated in Table 3, indicating good accuracy of the proposed HPLC method.

3.1.5. Precision. The values of %RSD for intraday and interday variation are given in Table 4. In both cases, %RSD values were found well within 2% limit, indicating that the current method is repeatable.

3.1.6. Sensitivity. The LOD and LOQ of vinpocetine by the proposed method were found 0.0968 μgmL^{-1} and 0.2904 μgmL^{-1}, respectively. Figure 3 shows the sensitivity of the current method.

3.1.7. Ruggedness. The results (% of Recovery ± Standard Deviation of six assay samples) are given in Table 5, indicating the ruggedness of the current method.

3.1.8. Robustness. The % of RSD of robustness testing under different altered conditions is given in Table 6, indicating that the current method is robust.

4. Conclusion

The developed RP-HPLC method for the determination of vinpocetine is simple, precise, accurate, reproducible, and highly sensitive. The developed method was validated based on USP and ICH guidelines [12, 13]. Hence, this method can be used for the routine determination of vinpocetine in pure and pharmaceutical formulations.

Acknowledgment

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