RADS-JPPS Vol 4 (1), June 2016, 28-33

Development and Validation of RP-HPLC Method for Quantitative Estimation of Vinpocetine in Intellan Capsule

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ABSTRACT

Simple, precise, cost-effective and accurate high performance liquid chromatographic method for the determination of vinpocetine in API (active pharmaceutical ingredient) and Intellan Capsule formulation has been developed and validated. Chromatography was carried out at ambient temperature on a prepacked Purospher Star, (5 mm, 250 x 4.6 mm) and Hibar 250-4.6 columns with the isocratic mobile phase of acetonitrile: Ammonium acetate (55:45 v/v) adjusting pH to 2.8. The UV detection was carried at 280 nm. The results obtained showed good agreement with the declared contents. Vinpocetine detected in less than 25 mins with good resolution and minimal tailing and without interference of excepients. The method was linear in the range of 5–100 µgmL-1 with a correlation co-efficient 0.999 (inter and intraday CV<2 .0%). The recovery was 99-102%. The method was validated according to ICH guidelines and the acceptance criteria for accuracy, precision, linearity, specificity and system suitability were met in all cases. The proposed method can be used for quantitative determination of Vinpocetine from API and formulations.

Keywords: *Vinpocetine, formulation, and RP-HPLC.*

INTRODUCTION

Vinpocetine is a vinca alkaloid, synthetic ethyl ester of apovincamine, obtained from the leaves of the Lesser Periwinkle (Vinca minor) [1]. Chemically (Figure 1), it is 14-ethoxycarbonyl-(3α, 16α-ethyl-14, 15-eburnamenine) molecular weight of 350.454 [2]with molecular formula of C22H26N2O2 .It has vasodilating activity and widely used for the treatment of acute and chronic stroke[3-5]. It also used in the treatment of Alzheimer's disease and Parkinson's disease[6]. Vinpocetine selectively inhibits voltage-sensitive Na+ channels, resulting in a dose-dependent decrease in evoked extracellular Ca++ ions in striatal nerve endings and also inhibits IKK preventing IκB degradation and the following translocation of NF-κB to the cell nucleus.

In recent years, the major focus of pharmaceutical research is pointing towards the development of herbal products for treatment. It is reported that Intellan treatment significantly improves memory functions and reduces depression in elderly patients by acting as an energizer.

Several method have been reported for the analysis of Vinpocetine in formulation and active but the developed method is easy simple, accurate, specific, cost-effective, and validated RP-HPLC according to USP and ICH guidelines[2, 7, 8].

The aim of study is to formulate Intellan Capsule using vinpocetin and evaluate by

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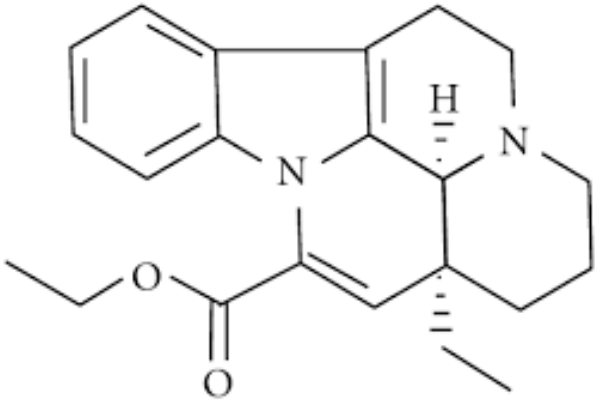
HPLC. This method used for the assessment



and routine analysis of vinpocetine in active and pharmaceutical formulations such as capsules and syrups.

Fig. 1: Structure of Vinpocetine

EXPERIMENTAL

Sample preparation

Pill out the powder of about 20 capsule and mix it . Take about 0.5 gm from this powder in a 10 ml volumetric flask and make up the volume with mobile phase. Sonicate the solution for 10 min using an ultrasonic bath and then filter through filter paper (Whatman 41).

Then Filter the resulting solution through a HPLC filter with pore size 0.45 µm and use filtrate for chromatography.

Standard solution preparation

Transfer approximately 10 mg (note exact weight) of USP vinpocetine standard in a 10 ml volumetric flask and dissolve in mobile phase, using an ultrasonic bath if necessary. Bring the solution's volume to the mark with mobile phase (1mg /ml).

Filter the obtained solution with pore size 0.45 µm and use filtrate for chromatography. Use only freshly prepared solution.

Analysis

Chromatograph 20 µL of test solution and vinpocetine standard solutions alternately on liquid chromatograph with UV detector or DAD detector obtaining not less than 6 chromatograms of standard and 3 chromotograms of sample solution in the following conditions:

Chromatography conditions:

High pressure liquid Chromatography with alternating UV or DAD detector.

Column

Chromatography column Purospher STAR LP RP-18 (5 µm) and Hibar 250-4.6, Merck or equivalent.

Mobile phase

Degassed mixture consisted of ACN: Ammonium acetate solution (55:45) (v/v)

Ammonium acetate solution preparation:

Weigh 15.4 gm of ammonium acetate and make up the volume with water up to 1 liter.

Flow rate – 1 ml/min.

Wavelength – 280 nm

Column temperature – Ambient temperature

Vinpocetine content in intellan with vinpocetine capsule calculated by the following formula:

$$X = \frac{A_{SPL} \text{ x } W_{STD} \text{ x } Dil_{SPL} \times \% \text{ purity}}{A_{STD} \text{ x } W_{SPL} \times Dil_{STD} \times 100} \text{ x M}$$

Where,

A_{SMP} – Mean value of peak area of tested solution samples

A_{STD} – Mean value of peak area of standard solution samples

W_{SMP} – Preparation weight, g

W_{STD} – Standard weight, mg

P – Percent Purity of standard sample

Dil_{SPL} - Dilution of sample

Dil_{STD} - Dilution of standard

M- average wt of capsule (0.5 gm)

Note: Vinpocetine should not be less than 2.5 mg/capsule.

RESULTS AND DISCUSSION:

The composition of intellan capsule is given in table 1.

Chromatographic condition

Initially, a Purospher Star, C18 (5 mm, 250 x 4.6 mm) column in isocratic mode, with mobile phase acetonitrile and Ammonium acetate in proportion of 55:45 (v/v) at a flow rate of 1.0 ml/min at a detection wavelength of 280 nm was used. To optimize the operating conditions for isocratic analysis using RP-LC detection, a number of parameters such as the mobile phase composition, pH and the flow rate were different. Various ratios (80:20, 70:30, 60:40, 50:50v/v) of acetonitrile and ammonium acetate was experienced as starting solvent for suitability study then acetonitrile and ammonium acetate having the above ratios was tried. The difference in the mobile phase leads to considerable changes in the chromatographic parameters, like capacity factor, peak symmetryand retention time. The pH effect showed that optimized conditions are reached when the pH value is 2.8, producing well determined and sharp peaks for Vinpocetine assayed. Henceforth, in the present method pH was adjusted to 2.8 using wavelength 280 nm. However, the peak shape and resolution were found to be good when the mobile phase comprising of the ACN: ammonium acetate having pH adjusted to 2.8 with phosphoric acid was used in the ratio of (55:45 v/v) at a flow rate of l.0 mLmin-1 (filtered through a 0.45 micron filter). Drug solutions were injected into the column at the concentration of 100 µgmL−1 and both elution pattern and resolution parameters were studied. Data for system suitability is given in table 2. The retention time for vinpocetine was found to be 24 minute meeting the resolution criteria specified in USP 2008. A typical chromatogram of test solution in API formulation and placebo is shown in figure 2.

METHOD VALIDATION

The newly developed method has been validated and holds well for the determination of drug in raw materials and dosage formulations. For validation of analytical methods, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use have recommended the accomplishment of system suitability, selectivity, specificity, linearity, accuracy test, precision, sensitivity, limit of detection and quantification of the method.

System suitability testing

Typical system suitability results are summarized in table 2, all the values for the system suitability parameters are within limits. The method was validated according to the ICH guidelines.

Selectivity and Specificity

The selectivity and specificity of the method was recognized through the study of resolution factor of the peak of vinpocetine from excepients. The method demonstrated good resolutions and was found to be free of interference from the excipients (Fig. 2) used in formulation products and thus, the method is specific for vinpocetine

Linearity

Vinpocetine showed linear calibration curves in the range of 5, 10, 25, 50 and100 µgmL-1 ($r^2 > 0.999$) with regression eq $y = 1983.9x - 1956.9$

Accuracy

The accuracy of the method was evaluated from the recovery results of spiked placebo samples. Appropriate portions of stock solution of Vinpocetine were spiked into blank placebo matrix to produce concentrations of 80, 100 and 120% of the theoretical concentration. Mean recovery of samples was 99.9- 100.9% for Vinpocetine (table 3).

Precision

Precision was determined by six replicate determinations of standard solution and the relative standard deviations were <2% for Vinpocetine in active and capsule. Method precision or intra-assay precision was performed by preparing six different samples involving different weightings. Each solution was injected in triplicate under the same conditions and the mean values of peak area responses for each solution were taken. Intermediate precision was performed by analyzing the samples on two different days (table 4) employing different instruments.

Robustness

Robustness of the proposed method was estimated by changing:(i) mobile phase composition from acetonitrile: water to acetonitrile: ammonium acetate (55:45 v/v); (ii) the pH (iii) the flow rate 1.0 ml/min. System suitability parameters in table 2 were found to be within acceptable limits.

Table 1: Composition - Intellan capsule

S. No	Ingredients	Quantity / Capsule
01.	Centella asiatica – Barhami booti	500.0 mg
02.	Coriandrum sativum – Dhaniya Khushk	200.0 mg
03.	*Amomum subulatum – Ilaichi kalan*	200.0 mg
04.	Emblica officinalis – Amla	400.0 mg
05.	Canscora decussata – Sankha Holi	75.00 mg
06.	Delphinium denudatum – Jadwar	20.00 mg
07.	Herpestis monniera – Jalneem	125.0 mg
08.	Lavandula angustifolia – Ustu-Khuddos	50.00 mg
09.	Glycyrrhiza glabra Extract – Mulethi Extract	50.00 mg
10.	Ginkgo biloba Extract	50.00 mg
Excipients		
11.	Methyl paraben	1.620 mg
12.	Propyl paraben	0.300 mg
13.	Potassium Sorbate	0.300 mg
14.	Citric acid	10.00 mg
15.	Talcum powder	180.0 mg
16.	Calcium Diphosphate	100.0 mg
17.	Magnesium Stearate	2.500 mg
18.	Avicel PH 102 (Microcrystalline Cellulose)	25.00 mg

CONCLUSIONS

In short, our method is specific, sensitive, rapid and easy to perform for determination of vinpocetine in active and formulation. The limit of quantification, small sample volume of this method makes it advantageous for adaptation to routine assay requirements and enables determination of vinpocetine because of good separation of the chromatographic peaks. The obtained results are in good agreement with the declared contents of dosage formulations. Results are accurate and precise and are confirmed by the statistical parameters.

Each 500 mg Capsule Contains Extract From:

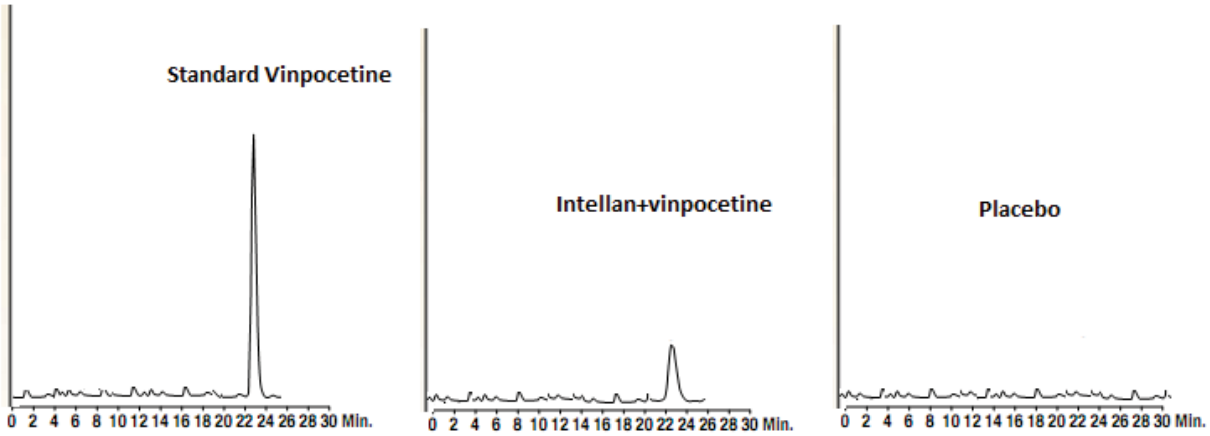


Figure 2: Chromatogram of Vinpocetine standard, in dosage form and placebo

Table 2: System suitability parameters

(% RSD)						
Parameters →	Retention time(Rt)	Capacity factors(K')	Theoretical plates(N)	Tailing factor(T)	Resolution (R)	Separation factor
Vinpocetine	0.421	0	0.71	0.607	0.415	0.68
Vinpocetine formulation	0.439	0	0.722	0.786	0.419	0.74

Table 3: Accuracy of Vinpocetine formulation

D rugs	Conc%	%RSD	% Recovery
	80%	0.019	100.9
Vinpocetine formulation	100%	0.095	99.99
	120%	0.058	100.2
	80%	0.076	100.01

Table 4: Inter day and intraday precision of Vinpocetine

Drugs	Conc. Injected mg mL−1	Inter-day	Intra-day
		%RSD	%RSD
Vinpocetine	5	0.201	0.817
	10	0.203	0.337
	25	0.101	0.546
	50	0.305	0.577
	100	0.301	0.497
Vinpocetine formulation	5	0.05	0.22
	10	0.36	0.23
	25	0.65	0.25
	50	0.92	0.26
	100	0.26	0.29

Reliability, rapidness, simplicity, sensitivity, economical nature, good recovery and precision of this method give it advantage over the other reported methods.

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