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Assay of Desmopressin in nasal spray by HPLC – UV detector with one Isocratic pump and system set – up

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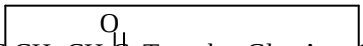
Abstract: *The aim of this study was to apply high performance liquid chromatography – UV detector to determine desmopressin in pharmaceutical preparation using one pump (isocratic pump) and ODS, 5um column. And Mobil phase acetonitrile: N, N diethyl formamide in ratio of 85:15 which show good quantitative and qualitative analysis in desmopressin acetate nasal spray (Cipla LTD INDIA) New controlled condition for setting-up the system to get a result with high resolution was shown in this study.*

Keywords- *desmopressin, HPLC method , isocratic pump and system set-up*

I. Introduction

Desmopressin acetate is a synthetic analogue of natural pituitary hormone 8-arginine vasopressin (ADH) antidiuretic hormone it is chemically defined as fallows.



S CH_2 CH_2C–Tyr–phe–Gln–Asn–Cys–Pro–D–Arg–Gly–NH_2. CH_3COOH. $3H_2O$
1-(3-mercapto propionic acid) 8-D arginine vasopressin mono acetate (salt) trihydarate
Desmopressin acetate is a large molecule with molecular weight 1183.34 and empirical formula C46H64N14O12S2.C2 H4O2.3H2O
Also we can conceder desmopressin as poly peptide molecule [(1)]
Desmopressin pharmaceutical preparations available in markets as tablet. powder and spray.

Desmopressin nasal spray can be used in these cases.
1- Vanwillebrands disease (inherited bleeding disorder due to defect in vanwillebrands factor)
2- diabetes insipidus (defect in urine concentration) .
3- hypopituitarism (decreased production of pituitary hormones)
Desmopressin is analogue of vasopressin desmopressin decreased vasopressor activity and increase anti-diuretic activity compare to vasoprossin.[(2,3)]
Desmopressin acetate preparations contain not less than 95% not more 105% of Desmopressin .
Many methods for assay of desmopressin were mentioned in Usp[(4)] like mass spectral analysis and retention time of major peak in chromatogram and specific rotation also microbial assay.
Enumeration test for specified micro organisms of amino acid concentration.
Determine the content of Desmopressin in pharmaceutical preparation by the biological method based on work of brsges and Thoron[(5,6,7)]
Biological activity of Desmopressin estimated by measuring the anti – diuretic effect of studied preparation compare with standard Desmopressin .
The possibility of replacement of biological method which determine the hormonal activity in the pharmaceutical preparation has been created by the introduction of high performance liquid chromatography to the qualitative and quantitative analysis of peptides.[(8)]
In Identification of multiple peptide and proteins (i.e. huge molecules) we must used HPLC with two pumps (binary pump) in order to increase the pressure of the mobile phase tobequite sufficient to more the huge molecules through the column. [(9)]
Jadwiga and wilcgynska[(10)] use HPLC with two pumps to determine the content of desmopressin in pharmaceutical preparation they performs the assay with mobile phase of PH=7 using column C8. 5 µm 100×4.6mm
This encourage use to use new technique in HPLC using one pump in assay of desmopressin by controlling all other HPLC parameters like maximum uv wave length, PH ,column, and mobile phase.

II. Apparatus:

1- Specord 40 (analytike Jena) (USA)
2- HPLC KNAUER (Germany) which consist of:

2.1- Isocratic pump 1000 KNAUER.
2.2- Degasser manager 5000 KNAUER.
2.3- UV detector 2500 KNAUER.
2.4- 250 × 4.6 mm Hyperclone 5u ods (C18) 120 A phenomenex column. (USA)
3- HP-TLC GAMAG (EMERCK KGaA) (Switzerland)
4- Filler device to produce water for HPLC (Whatman – England)

III. Materials and reagent:

1- Acetontrile (HPLC grade)
Gradient 240 mm for uv scharlab S.L made in Spain.
2- N-N dimethyl formamid for HPLC and spectroscopy Sd fine-CHEM limited MUMBAI-INDIA.
3- Water for HPLC by filter device.
4- NaOH Sodium Hydroxide. (AVONCHEM limited UK).
5- Desmopressin acetate stander.
Usp reference standard desmopressin acetate.
CAT. No 1173202 usp ROCKVILLE. MD LOT FOE 267.
6- Desmopressin acetate studied sample
Desmopressin acetate Nasal spray 0.01% w/v Cipla LTD INDIA.
7- Boric Acid (CHEM limited MUMBAI-INDIA).
8- Potassium Chloride BDH England.

IV. Method:

HPLC (KNAUER) with Isocratic pump (one pump) and (C18) 5u ODS column using Mobil phase of a mixture of acetonitrile and N, N diethyl formamid with proportion of (85:15) respectively of PH 10$\pm$0.5 the assay was carried out at maximum Wave length 230 nm.
The flows Rate of Mobil phase was 2ml/mint the sample and the stander of desmopressin acetate have concentration of 0.01 mg/ml.

Preparation of standard solution.

The content of vial of stander desmopressin acetate was dissolved with water of HPLC to get a concentration of 0.01 mg/ml of desmopressin acetate.

Preparation of studied sample

1ml of desmopressin spray (contain 0.01% w/w desmopressin acetate) was transferred to 10ml volumetric flask and completed with water of HPLC to the mark of the flask to get concentration of 0.01 mg/ml of desmopressin acetate.

V. Result and calculation

In assay of desmopressin nasal spray we used HPLC method although desmopressin containing peptide bonds it is not very large molecule like other polypeptieds and proteins this encourages us to use one pump (one Isocratic pump) for the assay with Hyperclone ODS (C18) column this column has a pore size 120 A^o which is enough for large molecules like desmopressin acetate molecule.

Both chromatographic monographs for stander desmopressin acetate (fig.1) and studied desmopressin acetate (fig.2) show a peak of desmopressin at a retention time of 1.133 mints.
To calculate the percent (%) of desmopressin in studied sample we used this equation.

$T/S * 100 = c\%$

C% = desmopressin acetate percent.
T = Area under the carve for desmopressin studied compound.
S = Area under the carve for desmopressin in stander.
The average result for t = 486284 and the average result for S = 496171.

$$C\% = \frac{486284}{496171} * 100 = 98\%$$

This result was com play with usp limit [4] to confirm our result to see the validity of this method we used the method used by Jadwige Dudkiewies [10] for determination of desmopressin acetate.
For this procedure we used the same apparatus we used in our experiment with Mobil phase acetonitrile and water in ratio 17:83.

And rate of flow 1.3 ml/mint and maximum wave length of absorption Lmax =220.
The volume of injection is 20 µl with standard and studied material concentration = 0.01mg/ml the chromatographic diagram of stander material (fig. 3) and studied material (fig.4) shows desmopressin acetate at retention time of 5.65 mint and 5.75 mint respectively.

s and t was calculated which are:
s =278548
t =285450

$$C\% = \frac{278548}{285450} * 100 = 97.5\%$$

This is also comply with usp [4].

VI. Preparation and HPLC system Set – up

1- Control Maximum wavelength absorption

In assay of protein or polypeptides we must control all the parameters we used in the assay like the maximum wavelength absorption (L max), the PH of Mobil phase, flow rate and the pressure in pump [9].
L max control and set-up
Desmopressin molecule consists of different amino acid and peptides bonds each amino acid has different L max (maximum wave length) of absorption [11] as in table-1.

To set-up our L max for our method of assay we do three experiment

1.1 Experiment 1

By using specord 40 (Analytik Jena) we make a transmition scan for stander desmopressin 0.01 mg/ml for scan mode from 190-300 nm the diagram of the transmition scan shows high absorption (lower transmition) at (L max 200 – 230nm) as shown in (fig.5).

1.2 Experiment 2

To see which wave length 200,210,220 and 230 nm can give us the best resolution and Isolation for desmopressin we used HP-TLC (CAMAG) using 10×20 cm TLC plate silica gel 60 F254 and twin trough chamber 20×20 cm.
Mobil phase acetonitril: N, N diethyl formamid in ratio of 85:15 respectively and we make scan to our desmopressin acetate 0.01 mg/ml stander from 200nm wave length to 230nm the result shows a best resolution for desmopressin acetate at wave length 230nm.
(Fig. 6, 7 and 8) shows peak resolution of desmopressin acetate at Lmax 210 nm, 220nm and 230 nm respectively.

1.3 Experiment 3

Many other experiment for assay of desmopressin they used L max 220nm in the assay so to see which L max is better for our method of assay we run our method for assay of desmopressin one time using L max 220nm and another time using L max 230nm. The higher resolution and the sharp peak of desmopressin was at Lmax 230 nm (Fig. 1) and the broad peak was at L max 220 nm (Fig. 9) .

2 – control and set-up the PH of Mobil phase.

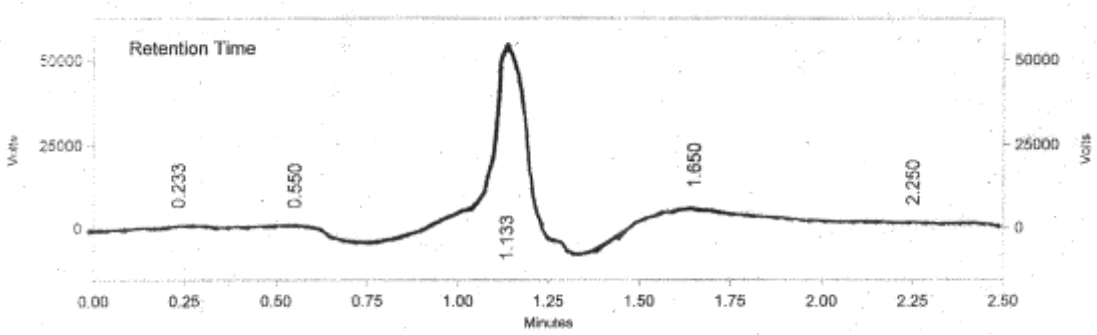
As our compound (desmopressin) consist of many amino acid and many peptides bonds and each amino acid has deferent pka [9].
We have to control the PH of Mobil phase which will give use the best resolution.
Experiment
We prepare alkaline borate buffer solution of PH 8, 9 and 10.
Method of preparation:
We dissolve 12.37g of boric acid and 14.19 g of potassium chloride (KCl in water and dilate to 2000ml to get 2 m solution.
50 ml of boric acid and KCl solution in 200 ml volumetric flask we add to it 3.9 ml 0.2 M NaOH complete the volume to 200 ml with water for HPLC we get Buffer solution of PH 8.
And also 50 ml of boric acid KCl solution in 200 ml volumetric flask and 20.8 ml of 0.2 M NaOH and complete the volume to 200 ml with water for HPLC to 200 ml we get buffer solution of PH 9.
Also we add 50ml of boric acid KCl solution in 200 ml volumetric flask and add to it 43.7ml 0.2 M NaOH and complete the volume to 200 ml with water for HPLC to 200 ml we get buffer solution of PH 10.
We used these buffer solution as a Mobil phase and we used the same HPLC system we used in our method with flow rate of 1.3ml/mint and we inject desmopressin 0.01 mg/ml stander solution with deferent Mobil phase of different PH in order to see the high resolution of assay of desmopressin.

We find that the best peak of height resolution is that when the buffer solution of PH 10 used as Mobil phase. (Fig. 10) shows the peak of desmopressin of PH 9, and (fig. 11) shows the peak of desmopressin at PH 10.

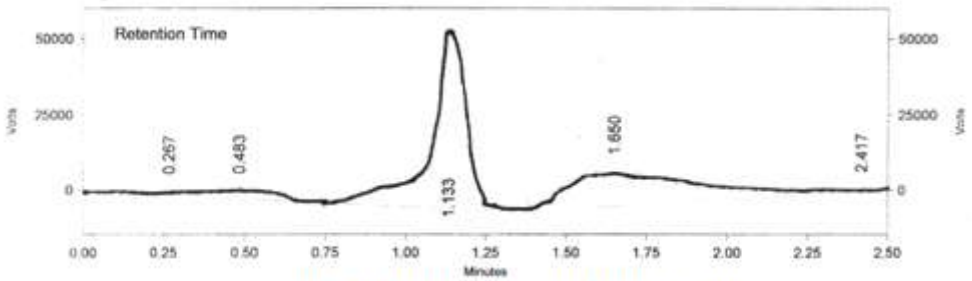
VII. Tables And Figures

Group	Wavelength (nm)
Peptide bond	190-210
His	211
Cys	250
Trp	280
Tyr	274
Phe	257

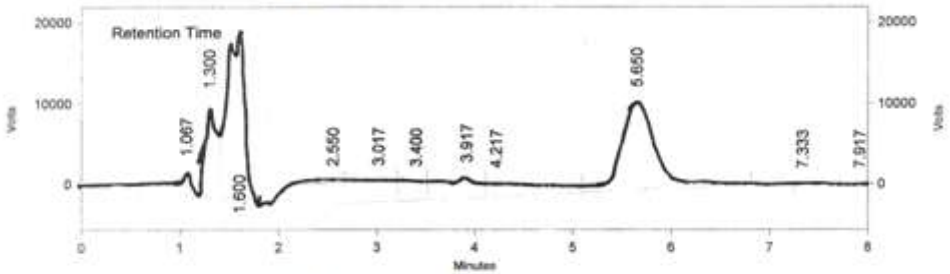
Table – 1 absorption bonds in protein (Campbell and Dwek 1984)[11]



(Fig-1) desmopressin acetate stander



(Fig-2) desmopressin sample (nasal spray)



(Fig-3) desmopressin acetate stander

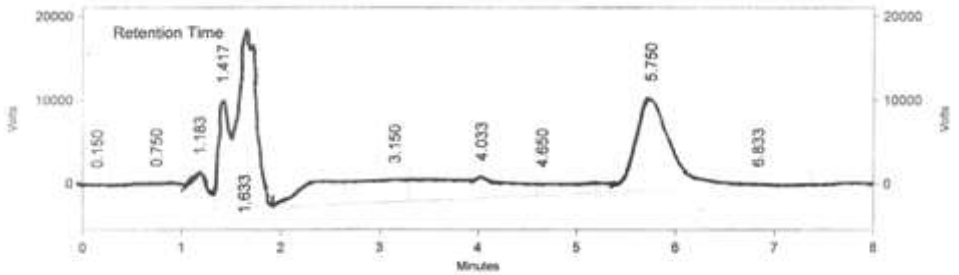
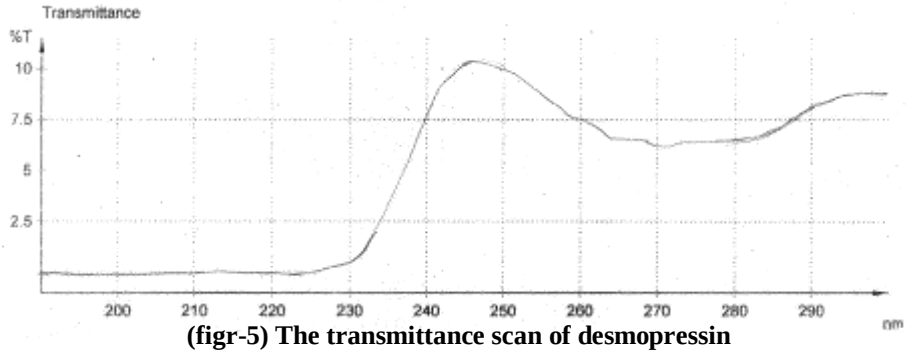
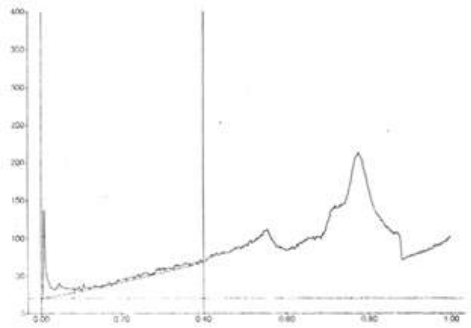


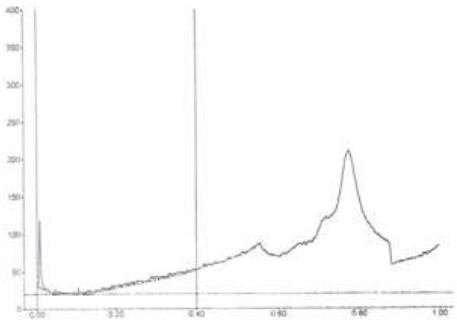
Fig-4 desmopressin Sample (nasal spray)



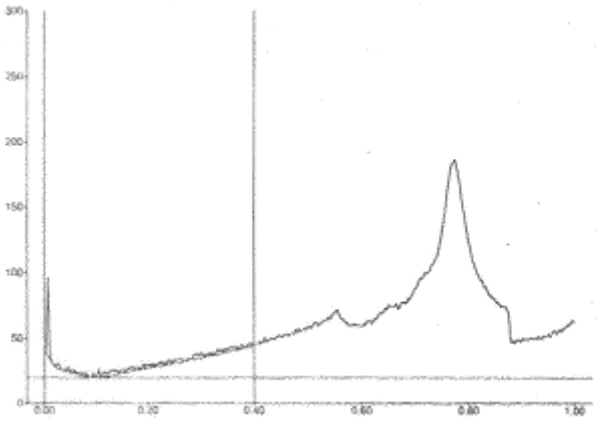
(figr-5) The transmittance scan of desmopressin



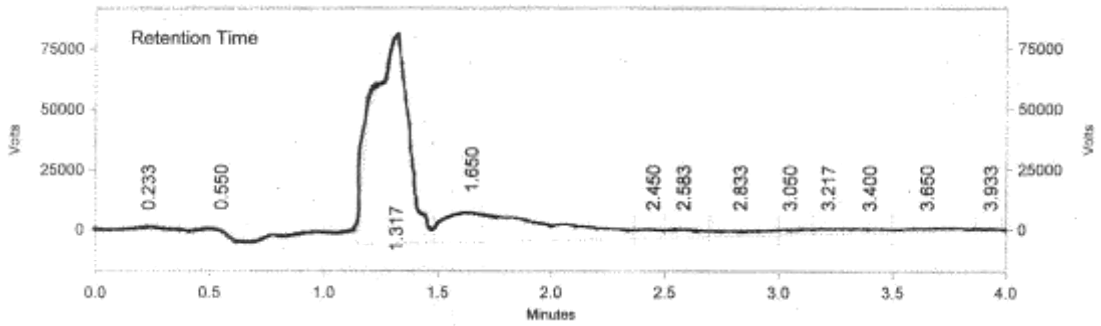
(Fig-6) Absorption at Lmax 210



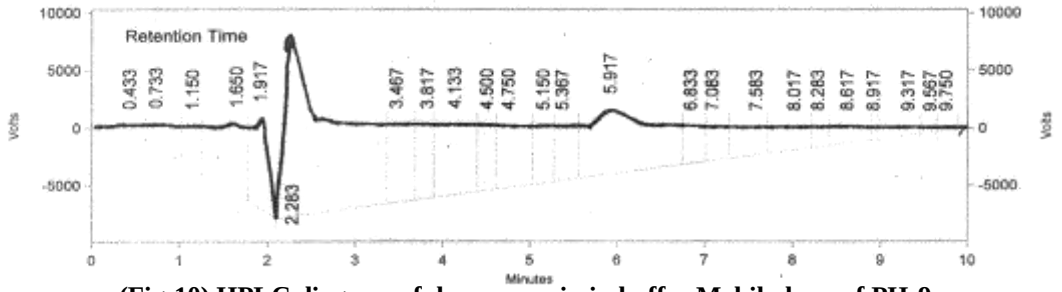
(Fig-7) absorption at Lmax 220



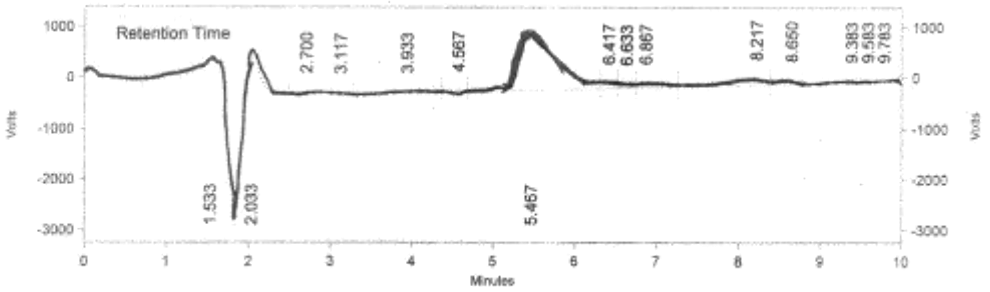
(Fig-8) absorption at Lmax 230



(Fig-9) HPLC diagram for desmopressin at Lmax 220 using acetonitrile: nn diethyl formamide (Mobil phase)



(Fig-10) HPLC diagram of desmopressin in buffer Mobil phase of PH-9



(Fig-11) HPLC diagram of desmopressin using Mobil phase buffer PH-10

VIII. Discussion & Conclusion

As we increase the percent of acetonitrile in Mobil phase the pressure in the purp will be decreases(8) so we increase the percent of acetonitrile in our experiment to 85% to get reduced in pressure.

This can help as to use one pump instead of two pumps to move the large molecules of desmopressin through our column with 120 A^o pores.

In our experiment we used the Mobil phase of acetonitrile: N,N diethyl formamide (85:15) this proportion give as the benefit over other HPLC methods.

1- The PH of this Mobil phase is 10 and as we see in PH set-up we get a best resolution in PH 10.
2- Reduced the pressure in our HPLC pump to 40 Mpa so we can use only one pump (Isocratic pump) instead of two pumps to move the large molecules of desmopressin through our column with 120 A^o pores.
3- Redaction of the pressure in pump make it easy to increase the rate of flow so the retention time will be short this can reduce the time of experiment and the loss of Mobil phase as we see in (fig.1,2,3,4).

In our new method of assay we can used less concentration of stander and tested material because this methods is very sensitive and of high resolution.

We can use in our method desmopressin consent ration of 0.01 mg/ml. While other method we used concentration not less than 0.25 mg/ml of desmopressin.

Although the Mobil phase in our method is expensive but we need less volume of Mobil phase, that the result to be achieved because we have less retention time for desmopressin acetate.

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