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RP HPLC determination of benzhexol hydrochloride in tablet formulations and urine

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ABSTRACT

A new, rapid and sensitive reverse phase HPLC method was developed and validated for the determination of benzhexol hydrochloride in tablet formulations and urine. The mobile phase used acetonitrile and water, (50 % v/v) adjust pH to 5 using phosphoric acid. The separation was achieved on C18 reversed-phase column (250 mm×4 mm i.d.). The flow rate was 0.6 ml/min and UV detection at 254nm. The retention time for benzhexol hydrochloride was 7.4 min. The calibration curve was linear up to 40μg/mL. The mean recovery for benzhexol hydrochloride is 101.16. The assay was precise within day and between days. The method provided excellent sensitivity, recovery, accuracy and reproducibility in therapeutic or toxic concentrations. Common excipients do not interfere.

KEYWORDS

Benzhexol hydrochloride;
Reverse phase HPLC;
C18 reversed-phase column.

INTRODUCTION

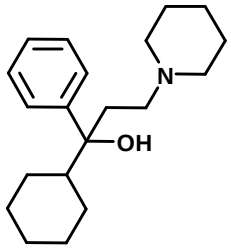
Benzhexol stops salivation and reduces skeletal muscle tone and rigidity in parkinsonian syndrome in which condition it is used to produce symptomatic relief and improvement of patient s condition[1]. Chemically, Benzhexol hydrochloride is 1-cyclohexyl-1-phenyl-3-piperidinopropan-1-ol hydrochloride, which has molecular formula C20H31 NO, HCl and molecular weight 337.9. It is a white or yellowish white crystalline powder of melting point about 250ºC. It is slightly soluble in water, sparingly soluble in alcohol and in dichloromethane, a 1% solution in water has a pH of 5.2 to 6.2[2].

Several high performance liquid chromatographic (HPLC) methods have been reported for the determination of benzhexol hydrochloride[3,4] and it's major metabolites. The present paper focuses on the use of an HPLC procedure based on a fast isocratic elution.

EXPERIMENTAL

Chemicals

Benzhexol hydrochloride(raw material) and Parkinol tablets(5 mg/tab) provided from El-nile company(provided from El-nile company, Egypt), phosphoric acid were purchased from Merck and acetonitrile HPLC Grade, methanol and chloroform were pro-



Benzhexol

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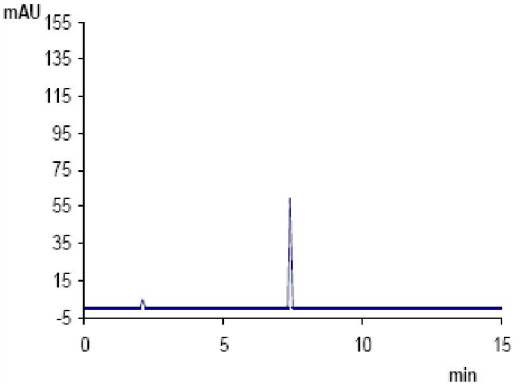

Figure 1 : Chromatogram of benzhexol(1 µg/mL)

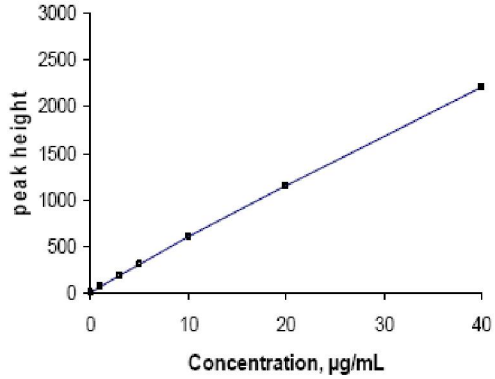

Figure 2 : standard calibration curve of benzhexol

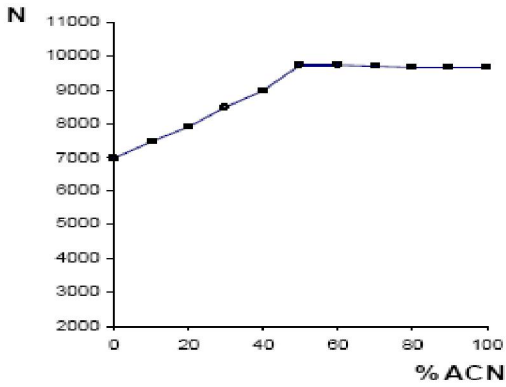

Figure 3 : Effect of the variation of acetonitrile composition on column efficiency

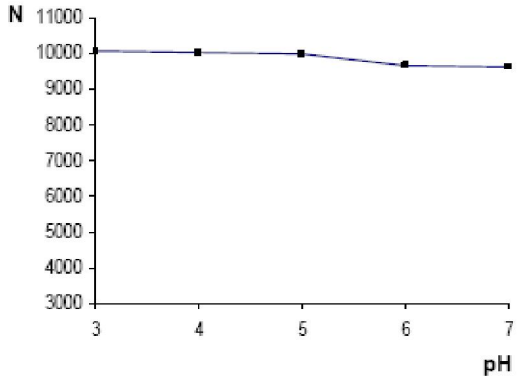

Figure 4 : Effect of the variation of pH on column efficiency

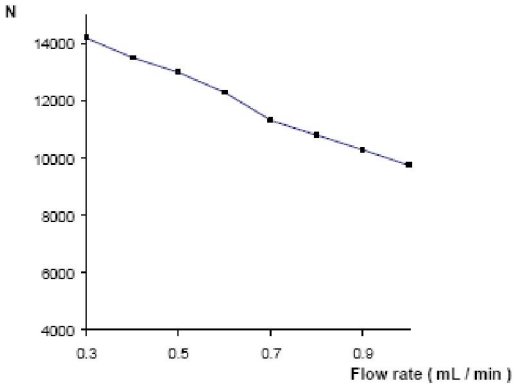

Figure 5 : Effect of the variation of flow rate on column efficiency

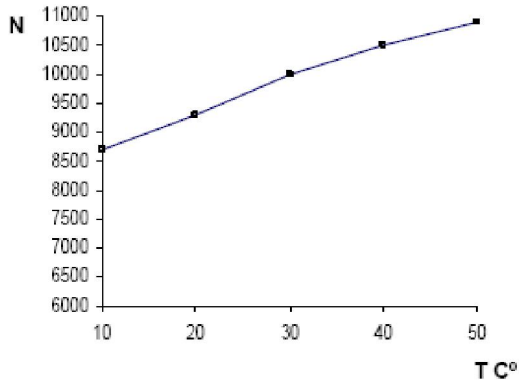

Figure 6 : Effect of the variation of temperature on column efficiency

vided from Sigma-Aldrich. Human urine was obtained from Medico-Legal Organization (Egypt).

Instrumentation and chromatographic procedure

A Hewlett-Packard "HP-1050 HPLC" instrument equipped with UV detector. An octadecyl silica "Li Chrosper 100 RP 18" column with 5µm particle size and ID of 250 mm×4 mm was used. The pH adjustment was carried out using Jenway pH-meter. The mobile phase of acetonitrile and water, (50 % v/v) adjust pH to 5 using phosphoric acid.

Sample preparation

Standard solutions

Ten tablets were weighted and the average tablet

TABLE 1 : Calibration data for benzhexol (n-6)

Range (µg/mL)	Coefficient of correlation r	Slope	Intercept
0.5-40 µg/mL	0.999	6.009	0.023

TABLE 3 : Chromatographic determinations of benzhexol in spiked urine samples

Added(µg/mL)	Found(µg/mL)	S.D.	Recovery (%) ± R.S.D.
10	9.50	0.06	95.00 ± 1.90
20	19.3	0.13	96.50 ± 3.61
30	28.5	0.07	95.00 ± 4.50

weight determined. The tablets were finely powdered and a portion of powder equivalent to one average tablet weight was weighted and quantitatively transferred into a 50 ml volumetric flask. 25 ml of methanol were added and the dispersion was shaken for 20 minutes. Ultrasonication followed for another 20 minutes and then the solution was diluted to volume with methanol shaked well and left to precipitate. Appropriate dilutions were made from the clear supernatant solution with acetonitrile so that in the middle of the standard solution range[5]. These solutions were stored at 4°C. Urine standard samples were prepared by dilution of the stock solutions with drug free urine.

Extraction

To 0.5 mL urine were added to a glass tube add 5 mL of chloroform(containing.benzhexol in concentration 0.5-40µg/mL). The tube was capped tightly. After vertical agitation for 2 min and centrifugation at 5000 rpm for 10 min, the upper organic phase was transferred to a clean conical tube and evaporated. The residue was reconstituted by adding 50µL of the mobile phase[6]. A total of 20µL was injected into the chromatographic system.

RESULTS AND DISCUSSION

Figure 1 shows a typical chromatogram obtained following analysis of benzhexol in tablets. Sharp and symmetrical peak was obtained with minimal tailing, thus facilitating accurate measurement of the peak area ratio. No interfering peaks were found in the chromatogram due to tablet excipients.

***to = 2.1 min**

where to is Void time(can be interpreted as part of the total analyte retention time that the analyte actually spends

TABLE 2 : Analysis of variance for intra- and inter day

Day/ Assay	1	2	3	4	5	6
1	4.99	5.02	5.07	5.05	4.96	5.03
2	5.01	5.03	5.05	5.02	4.95	5.04
3	5.03	5.02	4.97	5.03	5.01	5.02
4	4.94	5.01	4.99	4.93	4.96	4.92
5	Mean = 5.002					

in the mobile phase moving through the column, and for the rest of the retention time the analyte sits on the stationary phase surface).

Linearity

Detector response linearity was performed by preparing five triplicate calibration samples(0.5-40µg/mL) covering the range between therapeutic and toxic concentrations[7]. Calibration curves were obtained.

Precision and accuracy

Six replicate commercial tablets analyzed for Precision and accuracy. For the analysis of benzhexol in Parkinol tablets 5 mg, twenty tablets of each drug were weighed and finally powdered. A portion of the powder corresponding to 100µg/mL of the drug was weighed and dissolved in methanol and the flask was mechanically shaken for 5 min. The solution was removed into a centrifuge tube and centrifuged at 2500 rpm for 5 min Filter the solution and diluted by mobile phase. 20µL was injected. The injection occurred from the same solution during four days.

Six placebo tablets containing 5 mg of benzhexol.

The average recovery shown in Table 2 was 100.04 % with the coefficient of variation of 0.889 % Thus it was concluded that there was no significant difference for the assay which was tested within day and between days.

Assay detection limits

Limits of detection (LOD)

The limit of detection(LOD), defined as the lowest concentration of the analyte that can be clearly detected above the baseline signal, is estimated as three time the signal to noise ratio[8] LOD was detemined as 0.02µg/mL.

Limit of quantification (LOQ)

The LOQ was obtained by the same procedure

used for LOD, but estimated as ten times the signal to noise ratio. LOQ values were determined as 0.06µg/mL.

Ruggedness

The method ruggedness was tested by varying several chromatographic parameters and studying the effect on column efficiency (represented by the number of theoretical plates N).

Mobile phase composition

varying the acetonitrile percent from 0 to 100 % to choose the peter composition which give peter column efficiency.

Mobile phase pH

Varying the mobile phase pH between 3 to 7 to obtain better column efficiency. The results showed that at pH 5 column efficiency be higher and from pH 5 to pH 3 no significantly alter column efficiency.

Flow rate

variation of the flow rate from 0.3 to 1 mL/min, showed that column efficiency decreased when the flow rate increased. As the flow rate increased the retention time decreased so column efficiency decreased. However, 0.6 mL/min seems to be a good compromise when considering the chromatographic system and solvent economy.

Temperature

Varying the temperature between 10 and 50°C significantly altered column efficiency. Therefore a controlled temperature of 30°C was chosen.

CONCLUSION

This HPLC procedure appears rapid, simple, and suitable for routine analysis. Satisfactory validation data were collected for linearity, precision, recovery and ruggedness, LOQ values allowed to measure therapeutic and toxic concentrations. Its results were in agreement with those of reference methods.

REFERENCES

[1] S.C.Sweetman, Ed., Martindale; The Extra Pharmacopoeia, The Pharmaceutical Press, London, 36th Ed., **(2009)**.

[2] A.C.Moffat, M.D.Osselton, B.Widdop; Clarke's Analysis of Drugs and Poisons, **(2004)**.

[3] M.Gergov, I.Ojanpera, E.Vuori; J.Chromatogr.B, **795(1)**, 41-53 **(2003)**.

[4] V.Capka, Y.Xu, Y.H.Chen; J-Pharm-Biomed-Anal., **21(3)**, 507-517 **(1999)**.

[5] J.E.Kountourellis, C.K.Markopoulou, J.A.Stratis; J.Anal.Lett., **26(10)**, 2171-2183 **(1993)**.

[6] R.M.Azzam, L.J.Notarianni, H.M.Ali; J.Chromatogr.B, **708**, 304-309 **(1998)**.

[7] D.M.Bliesner; Validating Chromatographic Methods, **(2006)**.

[8] J.Caporal-Gautier; S.T.P. Pharma Pratiques, **2(4)**, 227-239 **(1992)**.