Mar. Üniv. Ecz. Der.,
3 (2), 85-89 (1987)

J. Pharm. Univ. Mar.,
3 (2), 85-89 (1987)

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MEFENOKSALON'UN YÜKSEK BASINÇLI SIVI KROMATOGRAFİK MİKTAR TAYİNİ

HIGH-PRESSURE LIQUID CHROMATOGRAPHIC ASSAY OF MEPHENOXALONE

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SUMMARY

In this paper, a high-pressure liquid chromatographic method was developed for determination of mephenoxalone using clopamide as an internal standard. C_{18}-column was used; the mobile phase was a mixture of 0.01M ammonium phosphate dibasic and methanol (65:35). The peak area is linear (r=0.9995) over a 80-400 μg/ml concentration range.

ÖZET

Bu çalışmada, internal standart olarak klopamid kullanılarak mefenoksalon miktar tayini için yüksek basınçlı sıvı kromatografik bir metod geliştirildi. C_{18}-kolon ve mobil faz olarak 0.01M dibazik amonyum fosfat ve metanol (65:35) kullanıldı. Pik alanı 80-400 μg/ml konsantrasyon aralığında doğrusaldır (r=0.9995).

INTRODUCTION

Mephenoxalone, 5-[(o-methoxyphenoxy)methyl]-2-oxazolidinone (1), has threrapeutic utility as a muscle relaxant in anxiety and depressive states in human subjects (2).

Fluorometric, Radiometric (3) methods have been published for the determination of mephenoxalone in biological fluids of dogs.

This paper describes a new high-pressure liquid chromatographic method in which clopamide, 3-(aminosulfonyl)-4-chloro-N-

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(2,6-dimethy-1-piperidinyl) benzamide (4), is used as an internal standard.

EXPERIMENTAL

Reagents and chemicals: Mephenoxalone and clopamide (internal standard) were supplied by İlsan İlaç ve Hammaddeleri San. A.Ş. Istanbul, Turkey. All other chemicals were commercial analytical reagent grade exept for the distilled water which was double distilled, filtered through a 0.5 μm filter (millipore Ltd) before use. Methanol (HPLC grade) was purchased from Merck (Darmstadt, G.F.R.).

Apparatus

A modular high-pressure liquid chromatograph with a constant-flow pump (model 510, Waters Assoc. Milford, Mass), a valve-type injector (model U6K, Waters Assoc.), a variable-wavelenght UV dedector (model 481, Waters Assoc.) and a data module recorder (model 730, Water Assoc.) were used. A stainless steel column (3.9 mm x 30 cm) packed with porous 10-μm silica particles, to which a monomolecular layer of octadecylsilane is chemically bonded was obtained commercially (Waters Assoc. prepacked μ-Bondapak C_{18} column).

Chromatographic Conditions

A mobile phase 0.01 M $(NH_4)_2HPO_4$-CH_3OH (65:35) and a flow-rate of 0.9 ml/min. at 2400 psi was used. The column eluate was monitored at 254 nm with a sensitivity of 0.05 a.u.f.s. and chart speed of 2 cm/min. The volume of sample injected was 20 μl (Hamilton Syringe). The system was operated at room temparature. Under these conditions mephenoxalone and clopamide eluted with retention times of 7.1 and 9.8 min. respectively.

Reference Standard Solution

Accurately weigh 0.05 g mephenoxalone and 0.005 g clopamide and transfer to a 25-ml volumetric flask and dilute to the mark with methanol. Pipetting 1 ml of this solution transfer to a 10-ml volumetric flask and dilute with methanol.

Stock Mephenoxalone Solution

Accurately eigh 0.05 g mephenoxalone, transfer to a 25-ml volumetric flask and dilute to the mark with methanol.

Stock Internal Standard Solution

Accurately weigh 0.005 g clopamide, transfer to a 25-ml volumetric flask and dilute to the mark with methanol.

Standart Solution for Calibration Curve

Accurately pipet volumes 0.4, 0.5, 0.7, 0.9, 1, 1.5, 2 ml of stock mephenoxalone solution in 10-ml volumetric flasks. Add stock internal standard solution (1 ml) to each flask and dilute to the mark with methanol and inject 20-µl aliquots of each to the chromatograph.

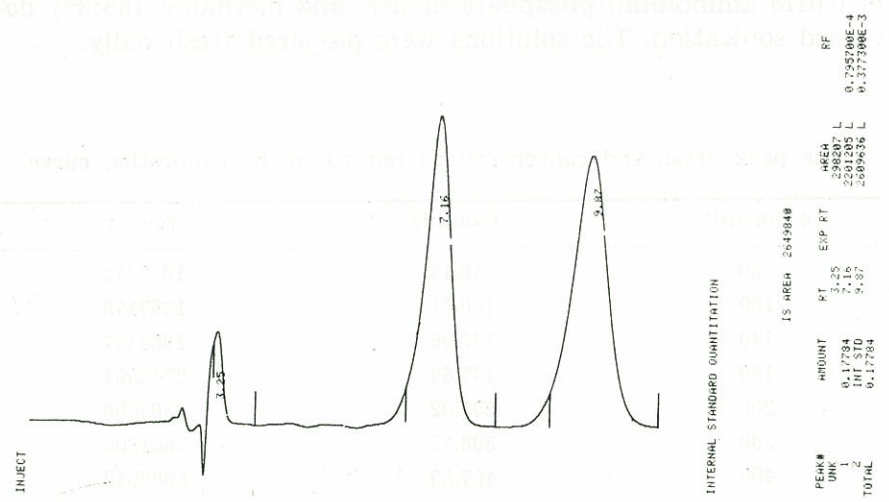

Fig. 1

Assay Procedure

Inject a 20-µl reference standard solution into the liquid chromatograph calculate the response factor. Then, inject a 20-µl each of standard solutions for calibration curve.

Mephenoxalone-Assay Procedure

Accurately weigh 0.05 g mephenoxalone bulk powder, transfer to a 25-ml volumetric flask and dilute to the mark with methanol pipetting 0.5 ml (100 µg/ml) of this solution transfer to a 10-ml

volumetric flask. 1 ml add of the stock internal standard solution to the flask and dilute with methanol. After calculating the response factor, inject 20-μ solution.

RESULTS AND DISCUSSION

A typical chromatogram of mephenoxalone and clopamide are shown in Fig. 1. The HPLC method takes less than 12 min. of chromatographic time to analyze one sample.

Mephenoxalone and clopamide were chromatographed at room temperature at a flow-rate of 2 ml/min. The mobile phase consisted of 0.01M ammonium phosphate dibasic and methanol (65:35) degassed sonication. The solutions were prepared fresh daily.

The peak areas and concentrations related to the calibration curve

c (μg/ml)	c (found) (x)	area (y)
80	78.13	1039212
100	100.61	1287178
140	142.96	1864457
180	177.60	2248991
200	201.02	2507608
300	308.37	3861898
400	407.50	5096512

y=bx+a	a=19148.5	b=12694.15	r=0.9995	n=7

To determine the linearity of the chromatographic response, a calibration curve was prepared in which the concentration of the internal standard was maintained constant while that of mephenoxalone was varied. The calibration curve was linear (r=0.9995) over a 80-400 μg/ml consentration range. The accuracy of the method is calculated by 10 determinations [mephenoxalone bulk powder, c=100 μg/ml (s=1.141)].

In summary, the HPLC method was reliable, reproducible, rapid and specific and should be useful for mephenoxalone determination.

ACKNOWLEDGMENTS

We are grateful to İlsan İlaç ve Hammeddeleri San. A. Ş. İstanbul, Turkey for supplying Mephenoxalone and Clopamide.

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(Received October 8, 1987)