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High Pressure Liquid Chromatographic Determination of Diethyltoluamide in Pharmaceutical Liquid Formulations

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HPLC에 의한 제제중 Diethyltoluamide의 定量

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=抄 錄=

방향성 방충제인 NN-Diethyl toluamide의 定量法은 NF의 IR法과 GC法만이 알려져 있으나, 著者等은 HPLC에 依한 分析을 試圖하여, 종래의 方法과 比較, 의의있는 結果를 얻었기에 보고하는 바이다.

INTRODUCTION

Diethyltoluamide (NN-Diethyl-methylbenzamide), an insect repellent agent, is supplied as liquid alone. Methods for the determination of diethyltoluamide include nitrogen determination by Kjeldahl method, I.R. spectrophotometry[1] and gas liquid chromatography[2].

However, these method do not have the rapidity, simplicity, sensitivity and accuracy of the high pressure liquid chromatographic methodology.

The purpose of these investigations is to develop a direct, simple and accurate method for the quantitation of diethyltoluamide in liquid pharmaceutical preparation.

EXPERIMENTAL

Apparatus—A high pressure liquid chromatograph, operated at ambient temperature, was equipped with a UV detector for the monitering the column effluent at 254 nm. The column was 30 cm×4 mm(i.d.) stainless steel packed with μ-Bondapak C_{18}. Samples were introduced through a septumless injector using a 10 μl syringe. The mobile phase was pumped at a pressure of 500 psig, which resulted in a flow rate of 0.5 ml/min.

Reagents—Diethyltoluamide NF reference standard was used. Reagent ACG grade methanol and distilled water were used in preparing the mobile phase. Mobile phase—The mobile phase was a mixture of methanol with distilled water (50:40).

Standard solution—Accurately weigh approximately 50 mg of diethyltoluamide reference standard, transfer quantitatively to a 50 ml volumetric flask and dissolve in and dilute to volume with the isopropylalcohol. Sample solution—Transfer an accurately weighed portion of the liquid equivalent to about 50 mg of diethyltoluamide to a glass-stoppered 50 ml volumetric flask and dilute to volume with isopropylalcohol. Chromatography—Condition the column for 24 hrs with the mobile phase at a flow rate of 0.5ml/min. This procedure is necessary for new columns, conditioning is not required for previously used columns. Inject 10 μl of the standard solution and adjust either the

pressure or flow rate so that the diethyltoluamide exhibits a retention time of about 10 min.

The approximate chromatographic conditions are a flow rate of 0.5 ml/min (at an inlet pressure of 500 psig), a chart speed of 12 in/hr and a detector sensitivity of 0.2 absorbance unit full scale (aufs). For the analysis of a sample, two 10 μl aliquots of the standard solution are injected followed by two 10 μl injections of the sample solution. The peak areas obtained or peak heights may be used for the calculations.

RESULTS AND DISCUSSION

Chromatographic Response—The typical response of diethyltoluamide to the chromatographic system is shown in Fig. 1. This chromatogram was obtained from the analysis of a quantity of diethyltoluamide liquid preparation containing diethyltoluamide 0.5 g in 1 ml.

A mixture of methanol with distilled water was used as mobile phase. To determine the linearity of the chromatographic response, a calibration curve was run in which the concentration of diethyltoluamide was varied (Fig. 1). The curve was linear

Table 1. Comparison of HPLC and Compendial Methods for Determination of Diethyltoluamide in Pharmaceutical Liquid Preparation.

Sample	Method of Analysis	
	HPLC(%)	NF XIV(%)
1	99.7	97.5
2	100.2	99.8
3	98.6	102.3
4	99.6	100.5
5	101.4	101.7
6	100.5	97.7
7	99.5	98.4
8	98.2	100.2
9	99.0	103.1
10	102.1	99.6
Mean	99.88	100.08
Variance	1.32	3.23

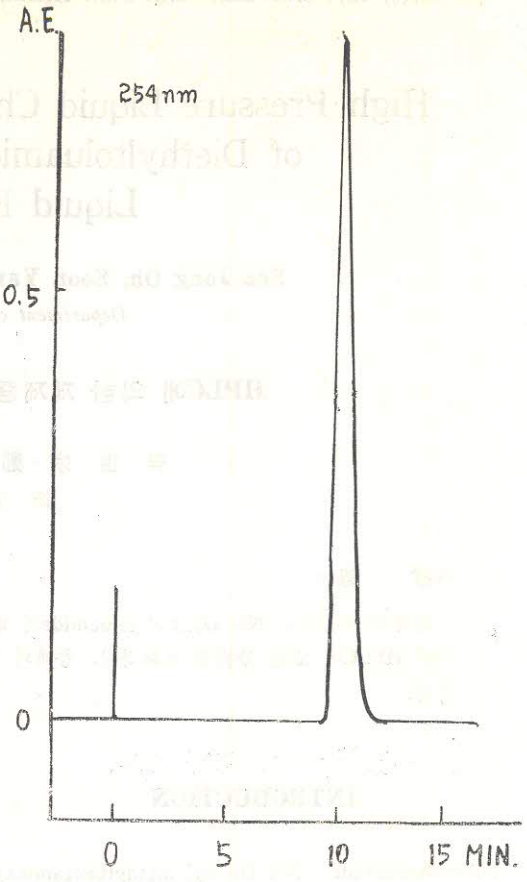

Fig. 1. Typical chromatogram of diethyltoluamide on μ-Bondapak C_{18} (Solvent system; MtOH:H_2O =50:40).

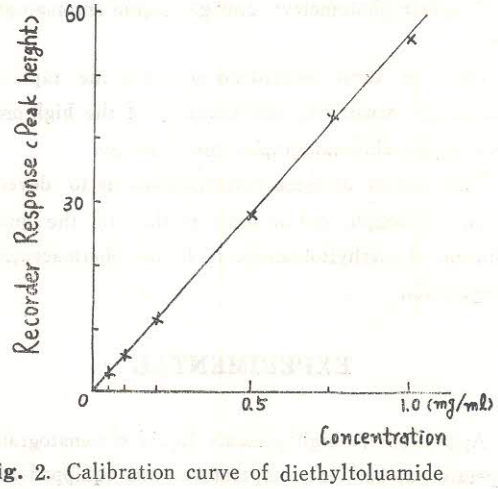

Fig. 2. Calibration curve of diethyltoluamide

(Fig. 2) over a 10 fold range, corresponding to quantities of diethyltoluamide that would be obtained

in the analysis of liquid preparations 20~200 % of labeled amount. The intercept was essentially zero.

Analysis of liquid pharmaceutical preparations Table 1 gives the results obtained from the analysis of a number of commercially available preparations containing diethyltoluamide. To verify the reliability of the method, portions of the same material taken for analysis by HPLC were also assayed using the method official in NF XIV (I.R. spectrometry). Estimates of precision were obtained using the analysis of variance statistical technique (Table 1).

The proposed HPLC method was compared to the compendial I.R. spectrometry, and the results were in agreement. All samples were within the requirements of official regulations. Because of the speed, accuracy and precision of the proposed procedure, it represents an alternative to present compendial methodology.

REFERENCES

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