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Studies on Determination of Aliphatic Carbamates

—Quantitative Analysis of Carisoprodol—

Jeoung Sook Kim and Bak-Kwang Kim

College of Pharmacy, Seoul National University

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For the determination of aliphatic carbamates, the quantitative analysis of carisoprodol was investigated by gas chromatography (GC) and spectrophotometry. All the methods studied were found to be very quantitative. The minimum experimental amounts of GC method, spectrophotometric method I and II were approximately 10^{-9}, 10^{-5} and 10^{-8} mole, respectively. The obtained results showed that GC method I was much more sensitive and rapid than spectrophotometric method II.

Propanediol dicarbamates, derivatives of the aliphatic carbamate, are the minor tranquilizer possessing tranquilizing and skeletal muscle relaxant properties.

The members of propanediol dicarbamate family are carisoprodol (N-isopropyl-2-methyl-2-propyl-1,3-propanediol dicarbamate), meprobamate (2-methyl-2-propyl-1,3-propaneodiol dicarbamate) and tybamate [2-(hydroxymethyl)-2-methyl pentyl butyl carbamate)], etc.

Carisoprodol produces muscle relaxation in animals by blocking interneuronal activity in the descending reticular formation and spinal cord, and is generally used as a skeletal muscle relaxant. Meprobamate is mainly used as a tranquilizer.

Because the effects of overdosage of these compounds, alcohol, CNS depressants or psycotropic agents are addictive and produce the physical dependence, these draw the important attention to the forensic region.[1)]

College of Pharmacy, Seoul National University

But their quantitative analytical studies have some difficulties because of their lack of UV absorption power and less reactive properties.

The quantitative analyses of these carbamate compounds are based on the chemistry of the carbamate moiety. They include acid hydrolysis followed by volumetric determination of the liberated ammonia.[2] Methods based on the acid catalyzed condensation with an aldehyde[3,4] and the ability of hypochlorite to N-chlorinate unsubstituted carbamate nitrogen[5] have been reported. Besides, several gas chromatographic(GC) methods have been proposed by using the derivative form of support.[6~9] Spectral methods based on infared(IR), near IR and nuclear magnetic resornance absorption also have been reported.[10~12]

In this paper, we investigated the specific and rapid quantitative analytical methods to determine carisoprodol by GC method and spectrophotometry for the routine analysis.

Experimental

Apparatus—The capillary gas chromatograph(Shimadzu) was equipped with a flame-ionization detector and a recorder.

The capillary column (20mL×0.3mm id) coated with OV-1 on sillanox was heated isothermally at 140°C. The injector temperature was maintained at 250°C. The detector temperature was maintained at 270°C. The flow rate of nitrogen carrier gas was 38ml/min. The spectrophotometer (Pye-Unicam) equipped with a Pye-Unicam AR-25 linear recorder was used.

Reagents and Chemicals—Carisoprodol and *p*-dimethylaminobenzaldehyde (DAB) were provided from Korea McNeil, Ltd. Trifluoroacetic anhydride (TFAA) and antimony trichloride used were of reagent grade. All other solvents used in this work were of reagent grade.

Antimony trichloride-acetic anhydride (ATA) reagent; An aqueous stock solution of antimony trichloride (ca. 25% w/v) saturated with chloroform is first prepared by warming on a hot plate, cooled and then filtered. ATA reagent is composed of 4 parts of this stock solution and 1 part of acetic anhydride. This reagent is stable for two days if it is stored under refrigeration.

p-Dimethylaminobenzaldehyde (DAB) reagent; 1 (w/v%) solution of DAB is prepared by dissolving it in benzene. When stored in the refrigerator, this solution is stable for about one month.

Acetic acid-acetone (AAA) reagent is composed of 3 parts of acetone and 1 part of glacial acetic acid.

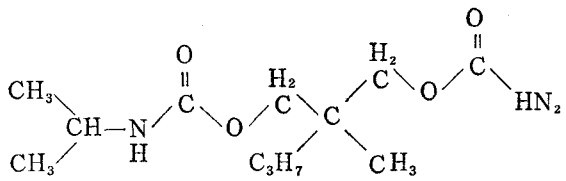
Procedure—GC method; 1.0mg of carisoprodol was transferred to a 10m*l* captube and 1.0m*l* of pyridine was added. After the addition of 100μl of TFAA, the tube was capped tightly and heated at 40~50°C for 30 minutes. The sample was conditioned by GC and analyzed by capillary GC. The injection volume was 1μl.

Spectrophotometric method I ; An accurately weighed amount (ca. 20mg) of carisoprodol was placed in 25m*l* volumetric flask together with 1m*l* of conc-HCl and completely dissolved with shaking. 3m*l* of 3% (w/v) DAB solution was added with mixing. Acetonitrile was added to volume and the solution was shaken sufficiently. The absorbance of reacted sample was measured at 420nm, using a reagent blank.

Spectrophotometric method II ; 10μg of carisoprodol was added to 0.5m*l* of AAA reagent, and then 0.5m*l* of 1% (w/w) DAB reagent was added and mixed well. 2m*l* of ATA reagent was pipetted into the tube. The contents were mixed, the stopper was inserted and heated immediately at 50°C for 10 minutes. The mixture was allowed to cool and diluted with 2m*l* of benzene. The absorbance of reacted sample was measured at 550nm, using a reagent blank.

Results and Discussion

GC method—Carbamates have been analyzed both as derivatives of the intact molecule and as derivatives of the products resulting from hydrolysis.[13] We investigated two aspects to provide the analysis of aliphatic carbamates using GC and spectroscopy.



Carisoprodol(M.W. 260.33)

The aliphatic carbamates are not "GC-able" under common columns because the carbamates are thermally unstable. For the GC-able form, the aliphatic carbamates need the deactivated column support, for example, AW(acid washed) or HP(high performance)

treatment. More effective analytical method is the acylation of aliphatic carbamates.[14] The acylation of intact carbamate may require additional steps in the analytical scheme, and such procedures exhibit significant unfavorable effects in the suppression of undesirable side effects of coextractives. Interference by coextractives is eliminated by this procedure. More characteristically, these derivatives possess shorter retention time, improved peak symmetry and increased stability to GC conditions than parent carbamates.

Table 1 shows the relationship between the reaction time and temperature for carisoprodol-TFAA derivatization. In this table, the optimum reaction condition was found to be 20 minutes at 60°C or 30 mins. at 40~50°C.

Figure 1 shows the relationship between reagent molar ratio and relative response. As shown in figure, the relative response increases as the reagent amount increases, but the relative response is almost constant when molar ratio exceeds 1 : 100.

Figure 2 shows the effects of hydrogen gas and air flow rate for the best response.

Table I—Reaction Condition for the Carisoprodol—TFAA Derivatization.

Reaction time (min.)	Temperature, °C			
	30	40	50	60
10	—	0.1601 (93.8)	0.1614 (94.8)	0.1638 (96.0)
20	0.153 (89.6)	0.1577 (92.4)	0.1575 (92.3)	0.1707 (100.0)
30	0.1535 (89.9)	0.1621 (95.0)	0.1624 (95.1)	0.1643 (96.3)
40	0.1553 (91.1)	0.1543 (90.4)	0.1609 (94.3)	—

( ) : Relative respone %

Figure 3 shows the relationship between sample concentration and relative response. This plot shows a straight line with a slope of 1.029 and correlation coefficient of 0.998 over the range of $1\sim10\times10^{-9}$ mole and supports the explanation of high quantitativity.

The effects of drug addition are shown in Figure 4. Phenacetine, prednisolone, phenylbutazone and caffeine are used as different drugs. As shown in Figure 4, the chromatograms of carisoprodol exhibit highly selective and sensitive peaks, but other drugs do not have any effects.

Spectrophotometric Method—The aliphatic carbamates have specific color reactions for the use of spectrophotometry by forming alcohol derivatives involved hydrolysis of

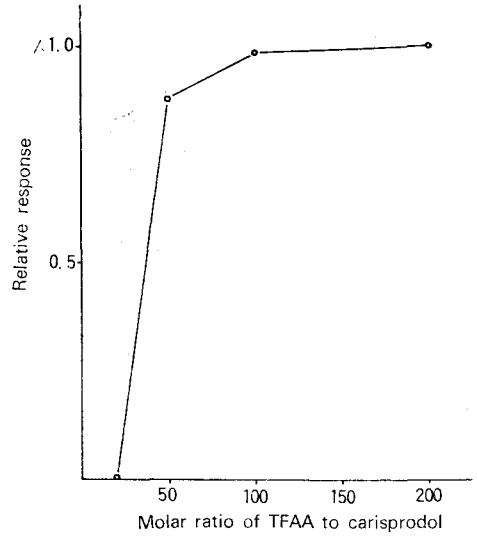


Figure 1—Effect of molar ratio of TFAA to carisoprodol on the derivatization.

the carbamate ester linkage. In this time, the chemical reaction between the aliphatic carbamate and aldehyde using methanol or acetone as solvent in the presence of concentrated acid is used in the laboratory region. But this method has been known to be less reproducible and less color-stable. This result is supposed to be attributed to solvent effects. With methanol, the blank have already some color and the color is increased with time

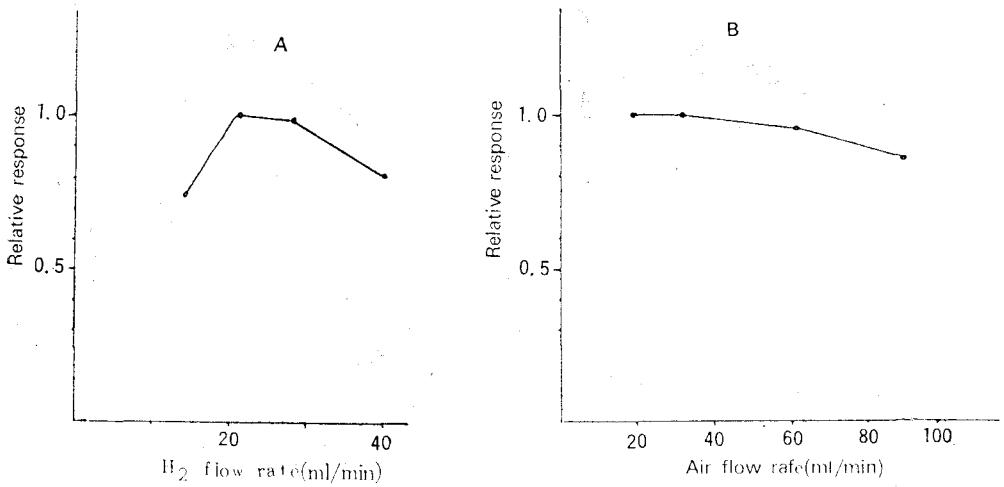


Figure 2—Effects of flow rate of hydrogen gas(A) and air(B) on the relative response.

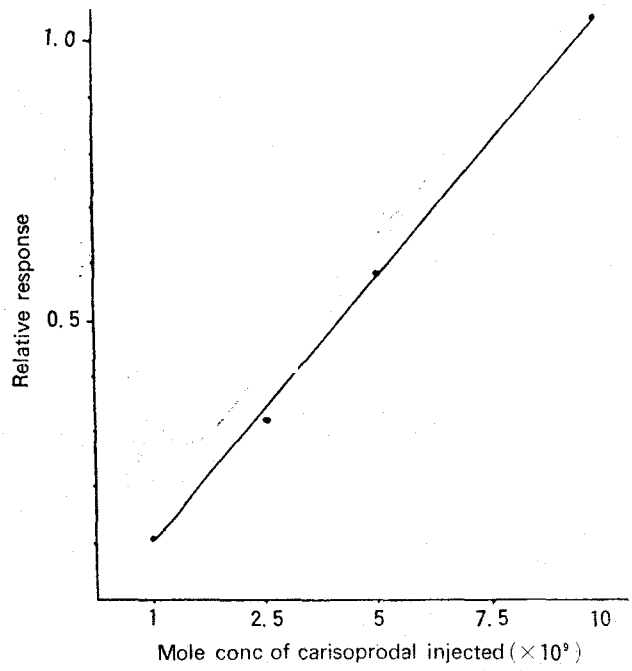


Figure 3—Relationship between sample concentration and relative response in chromatogram.

The result means that the solvent reacts with the reagent. And with acetone as a solvent, the color intensity is very low and bright green precipitates are produced with time. Therefore, we used acetonitrile as reaction solvent which is less UV absorptive, less reactive, pure and very stable in aldehyde and succeeded in producing the quantitative, reproducible and less changeable reaction product during the reaction time.

The absorption spectrum of reaction product obtained by spectrophotometric method I is shown in Figure 5.

Figure 6 shows that the absorbance is directly proportional to the concentration over the range of 0.4~1.6mg/ml of with a slope of 0.024 and correlation coefficient of 0.994.

Color intensity remains constant for at least one hour as shown in Figure 7. Meprobamate was studied in color development with certain aldehyde and anhydrous metallic salts in the presence of acid dehydrating agent. It was applied to the analysis of carisoprodol and the result was very accurate and capable of micro-analysis. The absorption spectrum of reaction product obtained from spectrophotometric method II is shown in Figure 8. This color reaction is specific for unsubstituted amides. The absorbances of colored solutions were measured at 550nm and were plotted over the range of 2~10μg with a slope of 0.046 and correlation coefficient of 0.99 as shown in Figure 9.

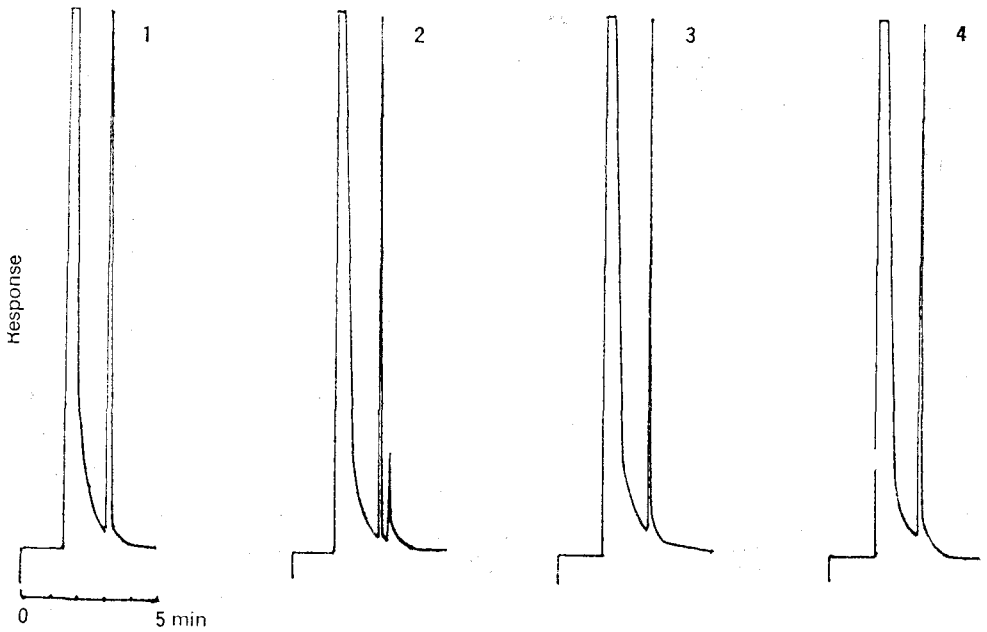


Figure 4—Other additive drug effects in GC chromatogram.
key : 1, carisoprodol; 2, carisoprodol+phenacetin; 3, carisoprodol+prednisolone; 4, carisoprodol+phenylbutazone+caffeine.

Some work has been reported which had described the application of high performance liquid chromatography(HPLC) directly to aromatic carbamate by using UV detector.[14,15]

We tried to apply this method to aliphatic carbamate, but failed because of lower de-

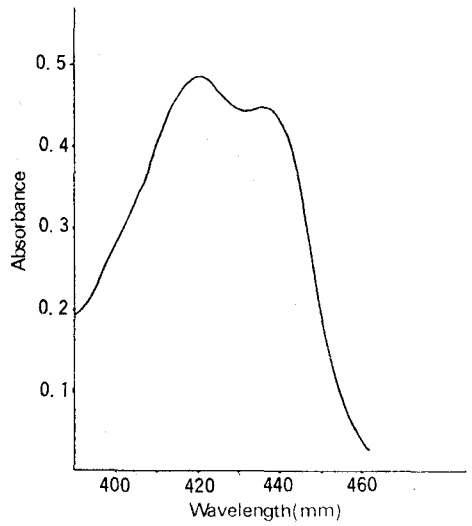


Figure 5—Absorption spectrum of reaction product by spectrophotometric method I.

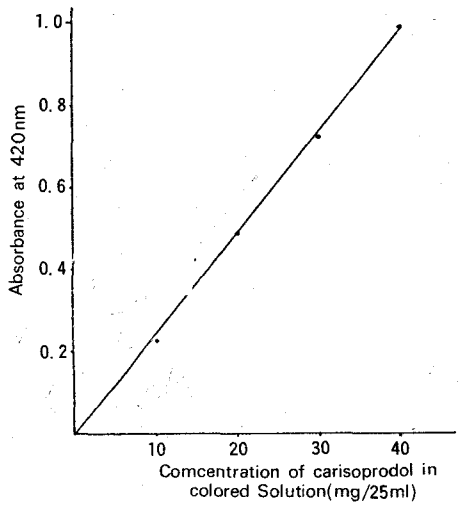


Figure 6—Calibration curve of reaction product by spectrophotometric method I.

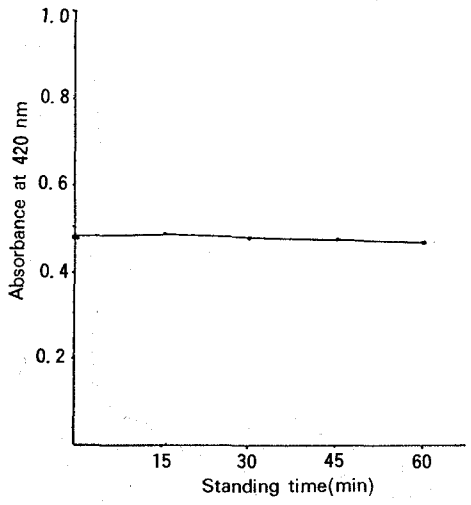


Figure 7—Effect of standing time on the color stability of reaction product in spectrophotometric method I.

tectability of aliphatic carbamates and the water resulting from the experiment.

We supposed that it might be the most important to avoid these interferences.

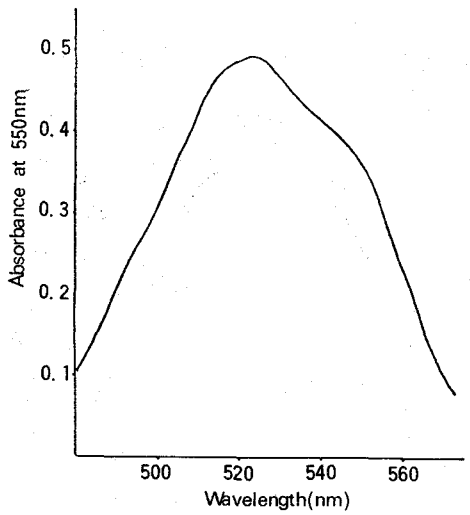


Figure 8—Absorption spectrum of reaction product by spectrophotometric method II.

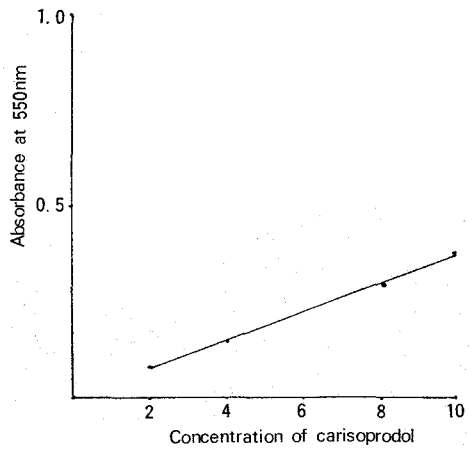


Figure 9—Calibration curve of reaction product by spectrophotometric method II.

Conclusion

1. The quantitativity of these methods was very accurate.

2. GC method enabled the experiment in the range of ca. 1×10^{-9}mole and in the retention time of 5 minutes.

3. Spectrophotometry by method I was very simple, but its sensitivity of measurement was lower than those in other methods.

4. Spectrophotometry by method II was very sensitive, but the procedure was so sophisticated.

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