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Spectrophotometric Determination of Pipethanate Hydrochloride in Pharmaceutical Preparations with Methyl Orange

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A new application of monoacidic dye is reported for the determination of pipethanate hydrochloride(PTH). The method is based on solvent extraction into chloroform of an ion-pair compound formed between methyl orange(MO) and PTH. PTH is determined by measuring absorbance of the extracts over the range of 2.0~12.0μg/ml at 420nm. Best accuracy can be obtained for solutions containing 3.0~8.0μg/ml. The color was stable for at least 5 days. The molar absorptivity was 2.88×10^4 l, mol^{-1}, cm^{-1}. Molar ratio and continuous variation plots have a maximum at 0.5 mol fraction of MO, indicating a 1 : 1 MO-PTH ratio

Pipethanate hydrochloride (PTH) is an anticholinergic agent used for relieving spasm of the gastrointestinal and urinary tracts.

Several reagents such as bromothymol blue(1-3), bromophenol blue(4), or bromocresol green(5) have been suggested for the spectrophotometric determination of onium compounds. However, in the case of such diprotic acids, the acidity gave a complicated effect on the extraction because stepwise dissociation occurred in the aqueous phase. Therefore, a singly charged tetrabromophenolphthalein ethylester (TBPE) has been used for the determination of various amines, alkaloids and quaternary ammonium salt as shown in previous papers(6-8). TBPE(6) is sensitively extractable as ion-pairs of quaternary ammonium salts or molecular complexes of some amines and alkaloids. In other words, TBPE has no selectivity because of the reaction with many onium compounds.

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However, 2,6-dichlorophenolindophenol(DCIP), which has one acidic group as well as TBPE, is able to form only a 1 : 1 complex with R_4N^+ (9-11). And R_4N^+ -DCIP ion-pair complexes are reproducibly extracted at pH 8.5 into nitrobenzene.

In the analysis of PTH(12), PTH-BCG complexes are extracted at pH 5.6 into chloroform and the extracts are treated with triethanolamine ethanol(1 : 1) mixture to develop a blue color which intensity is measured at 630 nm. However, this method presents some problems such as large dependence on the pH, unstability of PTH-BCG ion-pair complex, less reproducibility and complicated analytical procedure.

Also adsorption of dye complex on the dried filter paper and sodium sulfate anhydrous influences the analytical results to a large extent.

The proposed method with MO is, consequently, more suitable for the extraction in high selectivity, accuracy, no complication and only a little dependence on the pH, as compared with known methods.

Although nonaqueous titrimetric(13) and spectrophotometric methods were reported for the analysis of PTH, they are not appropriate for the analysis of PTH in mixed pharmaceutical preparations.

Also MO has been used as an indicator for acid-base titration.

This paper deals with the determination of PTH and its mixed pharmaceutical preparations.

Experimental

Instruments——Varian Techtron M 635 double-beam spectrophotometer with 1 cm cells, Beckman Zeromatic SS-3 pH meter, Mettler H-54 analytical balance, and Kookjae SH-SF shaker were used.

Materials and Reagents—PTH was supplied by Illyang Pharmaceutical Ind. Co., Ltd. dried at 105° for 2 hours.

MO (Matheson Coleman & Bell), BCG (Riedel-De Haen Ag Seelze-Hannover), chloroform and boric acid(Hayashi Pure Chemical Ind., Ltd), sodium sulfate anhydrous (Yoneyama Yakhin Kogyo Co., Ltd) and ethanol (Ishizu Pharmaceutical Co. Ltd.) were used. Other chemicals were of reagent grade or pharmaceutical grade.

0.1% MO in water; 0.1% BCG in 0.01 N sodium hydroxide in water;

Buffer solution—The McIlvaine buffer (pH 3.7) was prepared by mixing 0.2M sodium monohydrogen phosphate with 0.1M citric acid, followed by the adjustment of pH with 0.2M sodium monohydrogen phosphate or 0.1M citric acid; alcoholic boric acid solution(ABA)-1.3g of boric acid was dissolved in 100m*l* of ethanol by warming at 40°; standard PTH solution-a proper quantity of PTH was dissolved in distilled water and diluted to a concentration of 0.1%. The working standard solution with pH 3.7 buffer to a concentration required for the experiment. The

solution was prepared before using because of the unstability of PTH in aqueous solution, especially in alkaline region.

Assay Procedure—Pipet 10m*l* of buffer solution (pH 3.7), 3m*l* of standard PTH solution (3.2×10^{-4}M) and 3m*l* of 0.1% MO solution into a 50m*l* separating funnel.

Extract for 1 minute with 25m*l* of chloroform. After separation of two layers, pipet 15m*l* of yellow-colored extract into a 25m*l* volumetric flask, add 5m*l* of ABA solution, fill up to volume with ethanol, and mix well.

Measure the absorbance of the solution at 420nm, using a reagent blank or ethanol as a reference.

Results and Discussion

Absorption Spectra—In the presence of PTH, a yellow compound was extracted into chloroform, and the ion-pair complex had the maximum absorbance at 420nm as shown in Fig. 1, apparent molar absorptivity being 2.88×10^4 $1.Mol^{-1}.cm^{-1}$.

Effect of pH——The effect of pH on the extraction was studied by extracting PTH with MO from a series of aqueous solutions buffered to various pH values.

Fig. 2 revealed that the absorbance, of extracts was constant and maximum when the pH of the aqueous phase lay within the range of 3.0~4.0.

It was found that pH 3.7 was most suitable as the pH of buffer, as the pKa of MO was 3.7 and thus maximized the buffer capacity. And MO molecule was not extracted into chloroform even at lower pH. On the other hand, the optimum pH range for the constant and maximum extraction was found to be 2.0~4.0 for BCG-$CHCl_3$ systems as shown in Fig. 3. In this range of the pH, however, BCG molecule was considerably extracted into chloroform as well as BCG-PTH ionpair, and turned yellow with ethanol(Fig. 4), or blue with triethanolamine. Since the absorbance increased markedly with the buffer of lower pH, it was considered that it was impossible to use the buffer solution of lower pH than pH 5.2.

As the pH of buffer increased from the pH 5.2, the absorbance of the extracts decreased drastically and thus was very variable with the change of pH.

The difference of the optimum pH range for MO and BCG may be attributed to their different pKa values.

The pKa for BCG is 4.7 and that for MO is 3.7.

Accordingly, the pH range for the constant extraction of MO-PTH ion-pair was somewhat narrower than that for BCG-PTH ion-pair. When MO -PTH ion-pair was extracted from more acidic or alkaline solution than pH 3.0~4.0, the absorbance decreased because of precipitation or increasing dissociation of MO molecule, respectively.

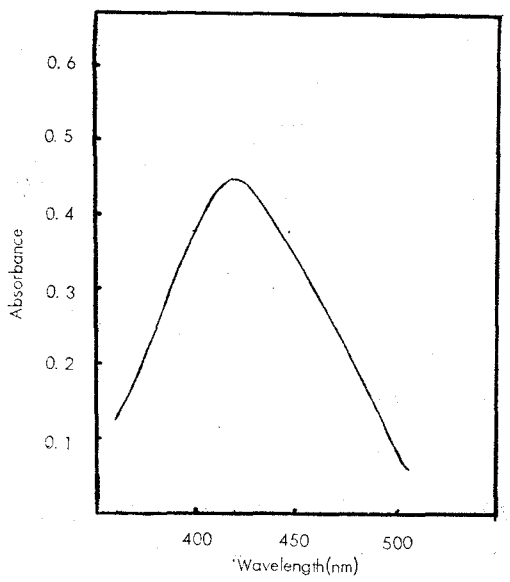


Figure 1—Absorption spectrum of methyl orange-pipethanate HCl ion-pair complex.

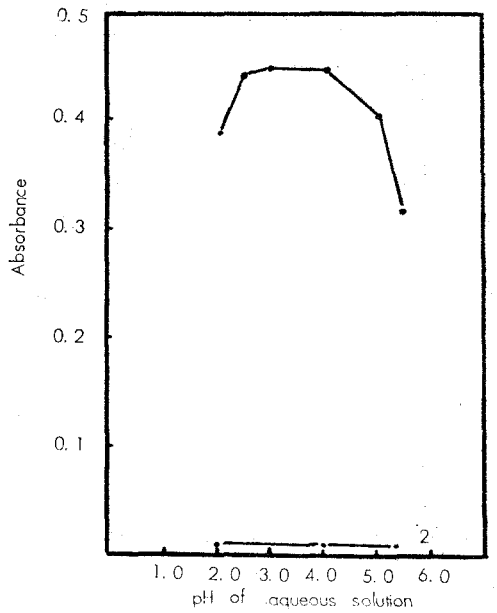
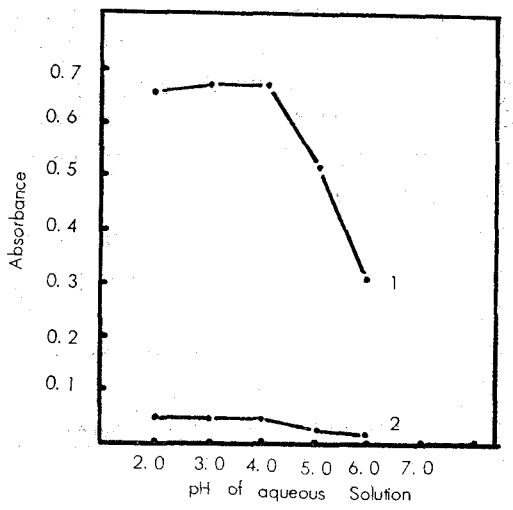


Figure 2—Effect of pH on the absorbance at 420nm. Key:1, extract with 1.6×10^{-5} M pipethanate HCl;
2, extract without pipethanate HCl. reference: chloroform



Fingre 3—Effect of pH on the absorbance at 420 nm in BCG method.
Key:1, extract with 1.6×10^{-5} M pipethanate HCl;
2, extract without pipethanate HCl. reference: chloroform

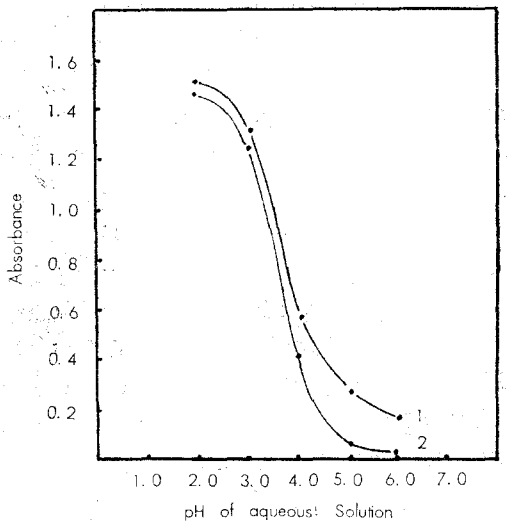


Figure 4—Effect of pH on the extractability of BCG molecule at 420 nm.
Key:1, extract with 1.6×10^{-5} M pipethanate HCl;
2, extract without pipethanate HCl. reference: chloroform

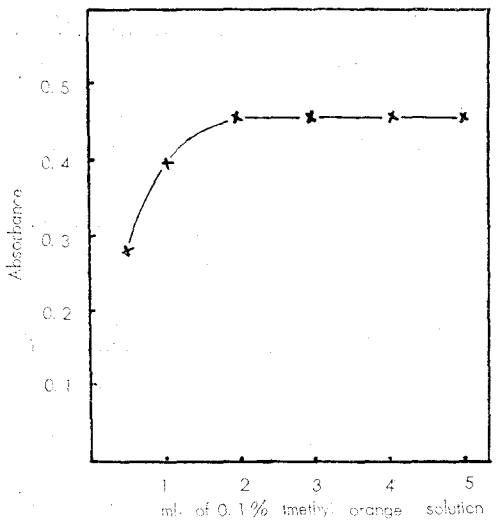


Figure 5—Effect of amount of 0.1% methyl orange solution on the absorbance at 420nm.

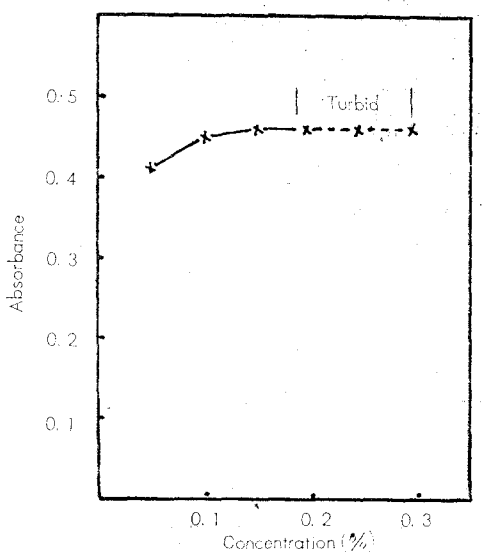


Figure 6—Effect of concentration of methyl orange on the absorbance at 420 nm.

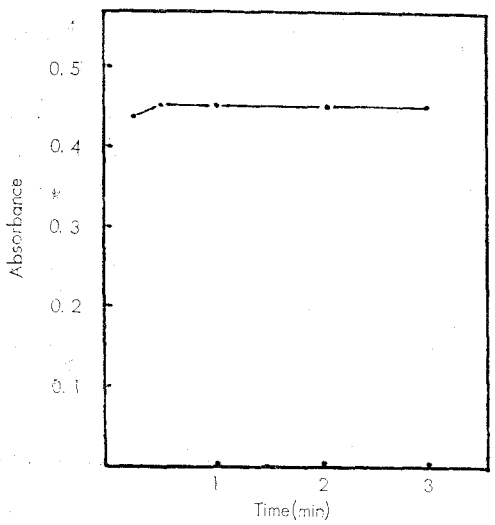


Figure 7—Effect of shaking time on the absorbance at 420 nm.

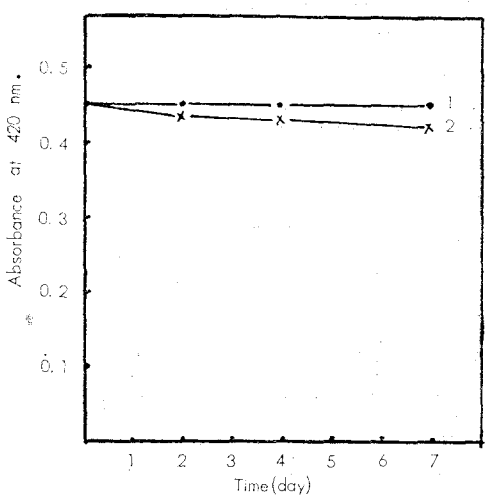


Figure 8—Stability of colored solution at 30°C.
Key : 1, chloroform-alcholic boric acid solution system(1 : 1);
2, chloroform.

Also when the chloroform layer was filtered or dehydrated with sodium sulfate anhydrous to clarify the solution, severe adsorption of MO-PTH ion-pair complex on filter paper or sodium sulfate was observed as shown in Table I. It was assumed that the extent of adsorption was dependent on the filtering area and moisture content of filter paper, or added amount of sodium sulfate anhydrous and dehydrating procedure.

Effect of Reagents——All other factors being kept constant, the effect of MO concentration was studied by adding MO solution of various volumes and concentrations. It was observed, as shown in Fig. 5, that the extraction of MO-PTH ion-pair was complete and constant above 2.0m*l* volume, but not complete at lower volume of the reagent.

When methyl orange of higher concentration in water than 2.0% was mixed with 10m*l* of pH 3.7 buffer, there were formed precipitates due to the decrease of solubility of MO in the medium. It was, therefore, favorable that at least 3 m*l* of 0.1% MO solution was added (Fig. 6).

When the buffer solution was used in the volume less than 5m*l*, a good separation of two layers was not done. Excess amounts (5~15m*l*) of the buffer soution used in the procedure had not any appreciable influence on the absorbance of the extract.

Effect of Other Parameters——Full color development with MO took place at the shaking time of 30 seconds.

Fig. 7. showed that continued shaking up to 3 minutes produced no further change in absorbance i.e., extraction.

It was, therefore, recommended that the mixture was shaken for at least 1 minute.

The color intensity of chlorform extracts added with ABA solution remained constant for at least 5 days, as shown in Fig. 8, but that of chloroform extracts of without ABA solution decreased significantly after standing for 1 day. By use of ABA solution, the stabilization of color intensity, and transparency of the solution were achieved.

It was observed that the chloroform extracts of MO-PTH ion-pairs were transparent in a range of 0.5~2.0% ABA solution, but not clear at higher concentration of the reagent because of the precipitation of boric acid in chloroform-ethanol system, as shown in Fig. 9.

Thus the concentration of ABA in ethanol was chosen 1.3%.

Fig. 10 and Fig. 11 revealed that reaction time and normal room temperature fluctuation caused no measurable effect on the extraction of MO-PTH ion-pair.

Composition of the Extracted Species——In order to clarify the composition of the extracted species, continuous variation and molar ratio plots were made at 420nm.

Fig. 12 and Fig. 13 showed that a 1 : 1 compound was formed between MO and PTH in the chloroform layer. Similarly, DCIP and TBPE, which are monoprotic acid dyes, formed a 1 : 1 compound as reported in the previous papers(8, 9).

Table I—Effect of Clarifying Methods on the Absorbance of Colored Solution

Conc. of Pipethanate HCl	3.2×10^{-5} M			1.6×10^{-5} M
Test Number	Intact Solution	Filtered Solution	Dehydrated Solution	ABA-treated Solution
1	0.903	0.666	0.703	0.450
2	0.891	0.604	0.637	0.447
3	0.895	0.642	0.658	0.451
4	0.908	0.715	0.605	0.457
5	0.883	0.724	0.598	0.443
6	0.912	0.674	0.706	0.450
7	0.907	0.640	0.601	0.452
Average	0.900	0.666	0.644	0.450
S.D.	0.0105	0.0427	0.0466	0.004
C.V.(%)	1.17	6.41	7.24	0.92

Table II—Reproducibility of Absorbance of Colored Solution Obtained from Standard Solution

Conc. of Pipethanate HCl	1.6×10^{-5} M
Sample	Absorbance at 420 nm
1	0.443
2	0.455
3	0.446
4	0.458
5	0.450
6	0.448
7	0.451
Average	0.450
S.D.	0.0052
C.V.(%)	1.14

Calibration Curve——The calibration curve obtained at pH 3.7 was linear over a range of 1.5~12.0μg/m*l* of PTH in colored solution(Fig. 14). In sevenfold determinations, the absorbance was 0.45 ± 0.0052, the relative standard deviation being 1.14% (Table Ⅱ).

Solvents and Colors of the Extracts——Table Ⅲ shows the colors of the extract by various solvents. In the case of isoamylalcohol with higher dielectric constant, red color appeared in the blank extract, which was considered to be caused from the

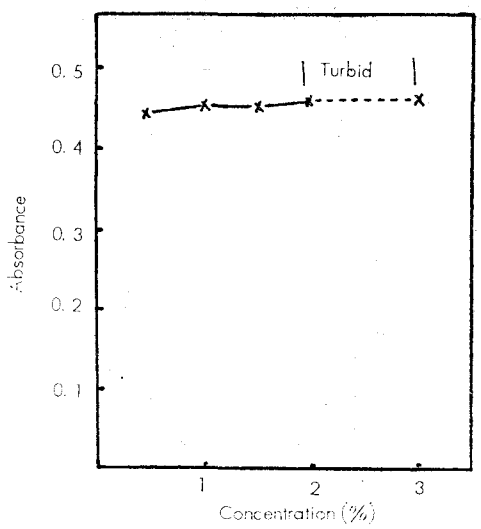


Figure 9—Effect of concentration of boric acid on the absorbance at 420 nm.

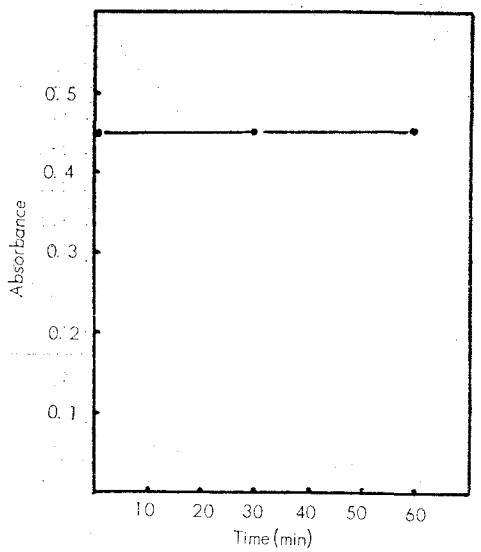


Figure 10—Effect of reaction time on the absorbance at 420 nm.

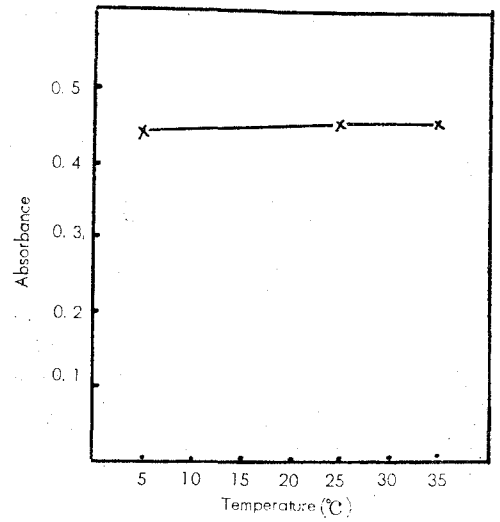


Figure 11—Effect of reaction temperature on the absorbance at 420 nm.

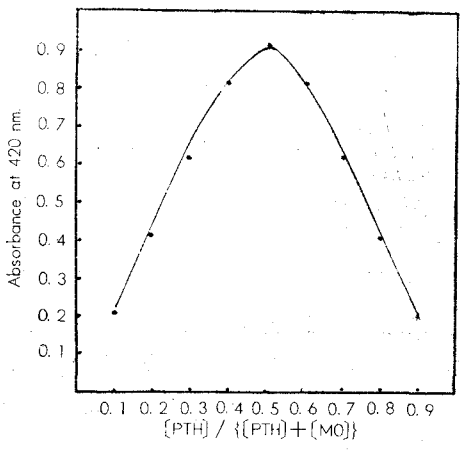


Figure 12—Continuous variation plots of pipethanate HCl to methyl orange

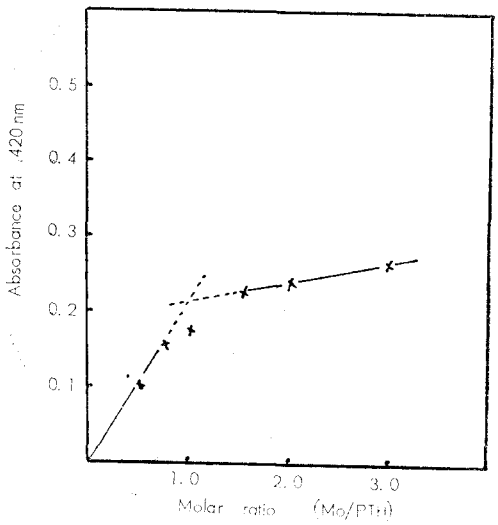


Figure 13—Molar ratio plots of methyl orange to pipethanate HCl.

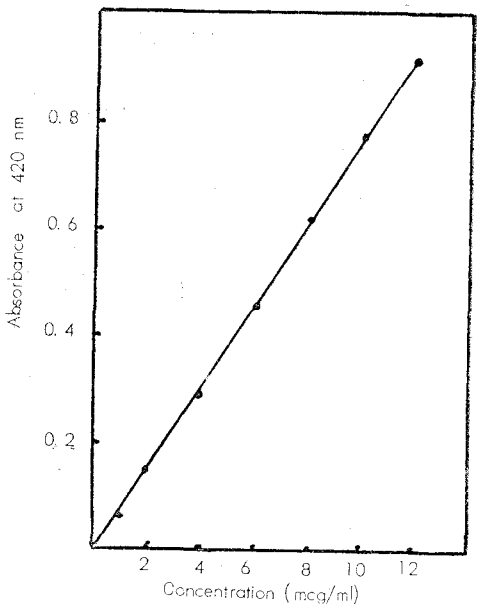


Figure 14—Calibration curve of pipethanate HCl.

dissociated species of MO.

On the other hand, in 1,2-dichloroethane, reagent blank showed a faint yellow color due to poor the extractability of MO molecule. By the presence of PTH, the yellow color developed in the solvents having low dielectric constant such as 1,2-

Table III—Colors of the Extracts with Solvents for Extraction

Solvent	Dielectric Constant	Boiling Point	Color	
			Blank	PTH
Nitrobenzene	34.38	210~211	yellow	yellow
Isoamylalcohol	14.70	132	faintly red	yellowish red
1,2-Dichloroethane	10.36	83.7	faintly yellow	yellow
Dichloromethane	9.08	39.95	colorless	yellow
Chlorobenzene	5.62	131~132	colorless	yellow
Chloroform	4.81	61~62	colorless	yellow
Toluene	2.38	110.6	colorless	in any case
Benzene	2.28	80.1		
Carbon tetrachloride	2.24	76.7		
Cyclohexane	2.02	80.7		
n-Hexane	1.89	69		

dichloroethane, dichloromethane, chlorobenzene and chloroform(Table Ⅳ). This may be attributed to the formation of a charge transfer complex by transition of the electron through the hydrogen bridge between the nitrogen of the base and oxygen of the dye.

Toluene, benzene, carbon tetrachloride, cyclohexane and n-hexane with lower dielectric constant did not extract the dye even in the presence of PTH.

Chloroform was found to be most suitable for extraction of MO-PTH ion-pair complex.

Effect of Foreign Substances——The effect of other foreign substances on the deter-

Table IV—Determination of Best Solvent for Extraction

Conc. of Pipethanate HCl	1.6×10^{-5} M Methyl Orange			
Solvent	Dye	λmax	Ab	As	ΔA
Chlorobenzene		420	0.010	0.065	0.055
1,2-Dichloroethane		420	0.040	0.491	0.451
Dichloromethane		420	0.025	0.497	0.472
Chloroform		420	0.008	0.458	0.450

Ab, Absorbance of reagent blank; As, Absorbance of sample.

Table V—Effect of Other Materials on the Determination of Pipethanate HCl

Other Material	mg/1.0 mg of Pipethanate HCl	Average Recovery(%)[1]
l-Glutamine	200	100.9
Sodium bicarbonate	50	94.5(98.3)[2]
Aluminum hydroxide	50	95.3(99.2)
Calcium carbonate, precipitated	100	98.5
Magnesium carbonate	100	99.7
Magnesium oxide	100	101.1
Glycine	10	98.9
Glycyrrhizin K_1	10	99.0
Hydroxypropylcellulose	15	107.2(100.6)
Polyvinylpyrrolidone	15	106.5(101.3)
Microcrystalline cellulose	100	101.0
Corn starch	100	99.3
Lactose	100	99.5
Magnesium stearate	15	100.2

1) Each result is the average of 3 determinations.
2) Extracted from weakly alkaline solution.

mination of PTH was investigated as shown in Table Ⅴ.

Sodium bicarbonate, aluminum hydroxide, hydroxypropylcellulose and polyvinyl pyrrolidon influenced the recovery of PTH significantly, but the considerable influences were removed by extracting PTH from weak alkaline solution with choloroform.

Table Ⅵ and Table Ⅶ show good results in the analysis of PTH in mixed artificial preparations and commercial products.

It was considered that MO method could be applied for the analysis of PTH in various products.

Table VI—Analytical Results of Pipethanate HCl in Mixed Artificial Preparations

Component	Declared, mg		
	A	B	C
Pipethanate HCl	3	3	1
l-Glutamine	600		200
Sodium bicarbonate		100	200
Aluminum hydroxide	100	100	100
Glycine	30		10
Glycyrrhizin K_1		30	10
Lactose	100		
Mannitol		100	
Microcrystalline cellulose	70		70
Corn starch	50		
Gelatin			25
Hydroxypropylcellulose	40		
Polyvinylpyrrolidone		40	
Carboxymethylcellulose Ca	10		
Magnesium stearate	7		
Found, mg	3.04	3.01	2.96
Recovery(%)*	101.3	100.3	98.7

* Each result is the average of 3 determinations.

Table VII—Analytical Results of Pipethanate HCl in Commercial Preparations by Proposed MO Method

Sample	Recovery(%)
Tablet A	101.2
B	99.4
Granule A	98.7
B	102.3
Powder A	97.5

Also MO reacted with various organic bases to form ion-pairs which were extracted into chloroform to develop yellow color (Table Ⅷ). It means that they can be determined by the organic extraction of their ion-pair complexes with MO.

Table VIII—Absorbancy of Various Organic Bases at 12 mcg/m*l* in Colored Solution by MO Method

Compounds	Mol. Wt.	λmax(nm)	Absorbance
Cetylpyridinium chloride	357.99	412	0.335
Chlorpheniramine maleate	390.87	420	0.753
Clemastine fumarate	459.97	418	0.668
Diphenhydramine HCl	291.82	420	1.086
Ephedrine HCl	201.70	408	0.032
dl-Methylephedrine HCl	215.73	420	0.340
Papaverine HCl	375.86	415	0.253
Phenylpropanolamine HCl	187.67	—	colorless
Pipethanate HCl	375.89	420	0.900
Strychnine HNO_3	397.42	420	0.742

Conclusions

The purpose of this investigation was to develop a quantitative spectrophotometric method for the determination of quaternary ammonium salt such as PTH with MO, and furthermore to apply this method to the analysis of pharmaceutical preparations. This method has the advantages of accuracy, simplicity and non-filtration over the known methods. Calibration curve for PTH was found to be linear over a range of 1.5~12μg/m*l* in colored solution of PTH. The standard deviation was 1.14% for PTH. The composition of MO-PTH ion-pair complexes was found to be 1 : 1 by continuous variation and molar ratio plots. The MO method for spectrophotometric determination of PTH gave the quantitative and reproducible results, and it was considered that MO could be applied as a dye complex forming reagent for the analysis of quaternary ammonium salts, amines and alkaloiods.

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