

Short Communication

Indirect determination of diphenhydramine hydrochloride by atomic absorption spectrometry

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Introduction

Recently, attention has been directed to the improvement of the sensitivity of analytical instrumental methods, in particular, those used for the determination of products of interest in pharmacology. Among such compounds is diphenhydramine, an antihistamine used for the treatment of a number of allergic reactions.

Several methods are available for the determination of diphenhydramine. In 1955, Graf and Friedler proposed a procedure for its determination in aqueous solution by titration with 0.05 N silicotungstic acid, using either methyl yellow or Congo red [1] as indicator. More recent methods, based on the ability of diphenhydramine to form complexes that are soluble in non-aqueous media and on its redox properties, involve treatment with Triton B in dimethylformamide [2] or iodometry, using 0.1 N sodium thiosulphate solution as titrating agent [3].

Since 1980 there has been a marked increase in the number of analytical methods that have been developed for determining diphenhydramine. This may be due to its increasing importance, because its antihistamine activity has been shown not to affect locomotive activity [4] nor possess mutagenic activity [5]. Instrumental methods for its direct determination include gas chromatography [6], liquid chromatography [7, 8], UV-vis spectrophotometry [9] and flow injection analysis [10].

The formation by diphenhydramine hydrochloride of coloured complexes that are extractable into organic solvents is the basis of several visible spectrophotometric methods. Coloured complexes are formed with vanadium and pyrocatechol [11], ammonium molybdate, potassium thiocyanate and lead chloride [12], cobalt(II) tetrathiocyanate [13] and with salts of zinc, cadmium and mercury(II) and pyrogallol or bromopyrogallol red [14]. Notwithstanding the many analytical procedures based on metal-complex formation and the extractability of the complexes into organic phases, none of the published methods employs atomic absorption spectrophotometry (AAS) for its determination. If such an organic extract is introduced into the flame of an atomic absorption spectrophotometer, the absorption will be an indirect measurement of the diphenhydramine concentration in the sample. Indirect procedures have been developed using iron(III) hexathiocyanate ($\text{Fe}(\text{SCN})_6^{3-}$) [15] and bismuth(III) tetraiodide (BiI_4) [16] as inorganic complexes which form an ion pair with diphenhydramine.

This paper reports an indirect determination of diphenhydramine hydrochloride, based on the formation of an ion pair with cobalt(II) tetrathiocyanate, extraction into 1,2-dichloroethane and AAS measurement of Co in the organic phase. The results are compared with those obtained by using iron hexathiocyanate and bismuth tetraiodide.

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Experimental

Equipment

A 370A Perkin–Elmer spectrophotometer (10 cm slit burner) with an air–acetylene (oxidizing-lean, blue) flame was used with the following settings: wavelength, 240.7 nm; lamp current, 37.0 mA; burner length, 10 cm; burner height, 100 mm; slit, 0.2 nm; air:acetylene ratio, 3.75:1. A Crison 501 pH meter was used for pH measurements. Whatman P/S paper (12.5 cm) was used to separate the phases.

Reagents

A standard solution of cobalt(II) tetrathiocyanate was prepared by dissolving 450 g ammonium thiocyanate and 218 g cobalt(II) nitrate in distilled water to give 800 ml of solution.

Aqueous solutions of diphenhydramine hydrochloride were prepared. All other reagents were of analytical reagent grade.

Procedure

Optimal conditions of cobalt tetrathiocyanate concentration, pH, shaking time, phase ratio and number of extractions were determined by mixing in 100-ml separating funnels with Teflon taps, a fixed amount of drug, different volumes of cobalt tetrathiocyanate stock solution, 0.1 M hydrochloric acid or sodium hydroxide solution to a determined pH and distilled water up to 50 ml. A 10 ml volume of 1,2-dichloroethane was then added,

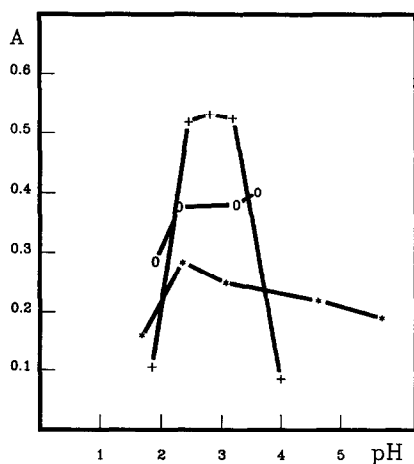


Figure 1
Effect of pH on the determination of diphenhydramine by AAS with $\text{Co}(\text{SCN})_4^{2-}$ (*), $\text{Fe}(\text{SCN})_6^{3-}$ (O) and BiI_4^- (+).

the mixture was shaken and the organic extract was filtered through P/S paper. The atomic absorption by cobalt in the organic phase was measured. Only one parameter at a time was varied.

The optimized procedure. Transfer an aliquot of sample solution containing 2 mg of diphenhydramine hydrochloride into a separating funnel. Add 30 ml of cobalt tetrathiocyanate solution, adjust the pH to 2.4 by adding an appropriate amount of sodium hydroxide (0.1 M) and dilute to 50 ml with distilled water. Extract with 10 ml of dichloroethane by shaking for 1 min and filter through Whatman P/S paper. Measure the absorbance under the analytical conditions described above.

Results and Discussion

Like other nitrogenous bases [17, 18], diphenhydramine hydrochloride reacts with cobalt tetrathiocyanate to form an ion pair which is blue in colour and insoluble in aqueous solution. The ion pair has a resinous appearance when solid, separates (into its constituents) at 35–40°C and undergoes polymerization. Consequently, it is unlikely to crystallize and, thus, cannot be used for the gravimetric determination of diphenhydramine.

The ion pair is soluble in chloroform and 1,2-dichloroethane, whereas the excess of the inorganic complex is not. Thus, the only species containing the metal in the organic phase is the diphenhydramine ion pair, which allows the indirect determination of the drug by AAS. For the reasons stated in previous work [18], 1,2-dichloroethane was chosen as the extraction solvent.

Figure 1 shows the effects of pH on the formation and extraction of the ion pair. The optimum pH range for the ion pair formed with cobalt thiocyanate is 2.3–3.5 in which range diphenhydramine exists as a protonated base which can associate with the negatively charged Co complex. Figure 1 also shows the influence of pH when iron hexathiocyanate and bismuth tetraiodide are used for the indirect determination of diphenhydramine.

The effect of cobalt tetrathiocyanate concentration on the ion pair formation is shown in Fig. 2. A wide concentration range (0.6–1 M) allows the maximum extraction of the ion pair. The extent of the formation of the ion pairs

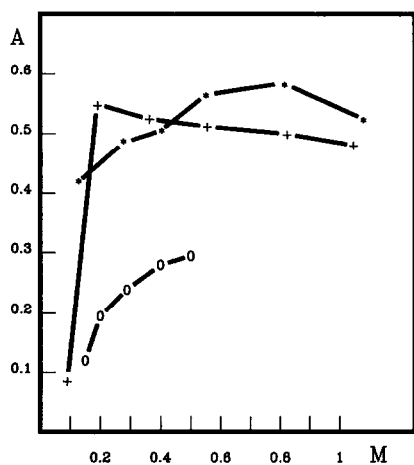


Figure 2
Effect of concentration of inorganic complex on the determination of diphenhydramine by AAS. $\text{Co}(\text{SCN})_4^{2-}$ (*), $\text{Fe}(\text{SCN})_6^{3-}$ (0) and $\text{BiI}_4^- \times 10^{-3}$ M (+).

with the Fe and Bi complexes is also shown in Fig. 2.

The shaking time was not found to be significant. Shaking times from 15 s to 3 min were studied and it was concluded that 1 min is sufficient to obtain constant absorbance values.

The stability of the extracts obtained was studied by preparing a stock solution of the extract and comparing its absorbance over a period of time to that of an extract freshly prepared under the same conditions. The extracts of the ion pair were found to be stable for 8 h.

Only one extraction was found to be necessary to obtain an ion pair recovery of almost 100%. When 10 determinations were made, the recovery was in the range 98.3–103.9%.

Linearity was obtained between 0.04 and 0.40 mg ml⁻¹ diphenhydramine in the organic phase and follows the equation: $y = 1.7833x -$

0.0631 where y is the absorbance and x is the concentration of diphenhydramine in the organic phase in mg ml⁻¹ with a correlation coefficient of 0.9994 ($n = 8$). The relative standard deviation for the determination was 1.70% ($n = 10$).

Values for sensitivity, characteristic mass and detection limit were determined according to IUPAC recommendations [19] and are shown in Table 1. These results show very good accuracy for the determination of diphenhydramine with the cobalt complex.

Comparative study of $\text{Fe}(\text{SCN})_6^{3-}$, BiI_4^- and $\text{Co}(\text{SCN})_4^{2-}$ complexes

Table 1 gives the optimum conditions for determining diphenhydramine hydrochloride by forming ion pairs with $\text{Fe}(\text{SCN})_6^{3-}$, BiI_4^- and $\text{Co}(\text{SCN})_4^{2-}$. The optimum pH in each case, was 2.5. However, the optimum concentration of the inorganic complex in the aqueous phase was different for each complex. A concentrated solution (0.9 N) of cobalt tetrathiocyanate was required whereas with BiI_4^- the optimum concentration was 0.18×10^{-3} M.

The linear range was greater with iron hexathiocyanate. However the linear range obtained with cobalt tetrathiocyanate was also sufficiently wide.

The best reproducibility was obtained with cobalt tetrathiocyanate, with which the relative standard deviation was 1.7% whereas that with BiI_4^- was 4.0%.

The effect of other substances on the accuracy of the diphenhydramine determination with cobalt was studied. No interference was observed with ephedrine, coramine, phenylbarbituric acid, glucose, guaiacol glyceryl ether or excipients at a ratio up to 10:1.

Other alkaloids and nitrogenous bases can

Table 1
Experimental conditions for the determination of diphenhydramine by AAS using different inorganic ions

Inorganic complex	$\text{Co}(\text{SCN})_4^{2-}$	$\text{Fe}(\text{SCN})_6^{3-}$	BiI_4^-
pH	2.4	2.5	2.6
Conc. of inorganic complex (M)	0.90	0.37	0.18×10^{-3}
Extraction number	1	1	1
Linear range (mg ml ⁻¹) in organic phase	0.04–0.40	0.05–0.7	0.01–0.1
Sensitivity (mg ⁻¹)*	0.187	0.05	0.679
Characteristic mass (mg)	0.28	0.11	0.04
Detection limit (mg)*	0.03	0.08	0.01
Relative standard deviation (%)	1.70	2.47	4.00

* Mass of diphenhydramine hydrochloride in the extraction tube.

interfere if they can be extracted as ion pairs. This occurs with amylocaine, atropine, avacine, spartaine, pilocarpine, procaine and quinine, but these substances are not co-formulated with diphenhydramine in pharmaceutical products.

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