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Determination of diphenhydramine hydrochloride in some single tertiary alkylamine pharmaceutical preparations by flow injection spectrophotometry

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Abstract

A simple and rapid spectrophotometric method with flow injection is proposed for determination of diphenhydramine hydrochloride in some single tertiary alkylamine pharmaceutical preparations (capsule and syrup). It is based on ion pair formation with bromocresol green in a pH 3 buffer which a yellow ion pair compound is extracted into chloroform layer. An aqueous layer containing excess bromocresol green is injected into a carrier stream of 0.01 M borax solution and absorbance of the stream is continuously monitored at 610 nm. Diminution of the bromocresol green is related to an amount of diphenhydramine hydrochloride that can be evaluated from a calibration graph established by a plot of diphenhydramine hydrochloride concentration (μg ml^{-1}) and peak height (mV). Optimization will be discussed. The calibration graph is linear over the concentration ranges of 5–21 and 75–188 μg ml^{-1}. A throughput of 100 injections h^{-1} can be obtained. Application to assay for capsule and syrup samples has been demonstrated. The method is validated by comparing the results with HPLC analyses.

Keywords: Diphenhydramine hydrochloride; Flow injection; Spectrophotometry; Single tertiary alkylamine pharmaceutical preparations; Bromocresol green

1. Introduction

Diphenhydramine hydrochloride, a histamine H_1-receptor antagonist is widely used as antiallergic, antiemetic and antitussive drug found in many pharmaceutical preparations. It is usually given orally in a preparation of tablet, capsule, or syrup. It may be administered by intramuscular or intravenous injection in severe allergies and ap-

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plied topically for local allergic reactions in preparations of lotion and cream containing 1–2% [1,2]. Several methods have been proposed for determining diphenhydramine hydrochloride in pharmaceutical preparations including titrimetry [3–5], fluorometry [6], electrochemical analysis [7] and spectrophotometry [8–11], which a batchwise ion-pair extraction has been applied [12–16]. Chromatographic methods have been used such as TLC-densitometry [17]; GC [18]; and HPLC [19]. The last one is used as an official method since 1990. However, those instruments are relatively high cost. Nevertheless, the titrimetric and spectrophotometric methods are time consuming. Flow injection (FI) procedures, which are rapid and automated, have been reported [20,21]. The procedures have some drawbacks such as precipitation in the line [20] or dealing with an uneasily controlled on-line solvent-extraction [21]. Bromocresol green has been reported for determining diphenhydramine hydrochloride by batchwise solvent extraction spectrophotometry [12]. Bromocresol green which is commonly available indicator in any laboratory is chosen for attempts in developing a simple FI procedure that does not require high cost equipments to overcome shortage of instruments in places where HPLC instrument is not available.

2. Experimental

2.1. Apparatus

A double beam spectrophotometer (Cary 1E, Varian, Australia) controlled by a CAF computer with Cary WinUV software (1 cm path length quartz cell and 2 nm fixed slit width) was used for absorption spectrum study.

An automated HPLC (Series 200, Perkin Elmer, USA) including IC pump, vacuum degasser, LC analytical column (Supelcosil LC-CN, 250×4.6 mm ID, 5 μm), guard column (Supelguard LC-CN), and UV/Vis detector (785A, Perkin Elmer, USA) controlled by a Digital Venturis 5100 computer with a chromatographic workstation software was used.

Recommended conditions used in the official method for determination of diphenhydramine hydrochloride by HPLC [19] were followed. Sample/standard solutions of the same volume were injected separately into the nitrile (CN) column using acetonitrile–water–triethylamine (50:50:0.5) solution as a mobile phase with a flow rate of 1 ml min^{-1} and detected at 254 nm.

2.2. Materials

All chemicals used were of analytical grade. Diphenhydramine hydrochloride reference standard (99.4% purity) was provided by the Royal Department of Medical Sciences, Thailand.

Some locally commercial pharmaceutical preparations (Table 3) containing diphenhydramine hydrochloride were taken as samples to be assayed.

2.3. Reagents and solutions

Deionized water was used for the preparation of all solutions.

Stock solution of diphenhydramine hydrochloride standard (1 mg ml^{-1}) was prepared by dissolving 0.1006 g of the standard in deionized water and diluting to the mark of 100 ml volumetric flask.

Working standards were freshly prepared by diluting the stock solution with deionized water to obtain appropriate concentrations.

Acid phthalate buffers of various pH values (range 2–5) were prepared using 0.2 M potassium biphthalate, 0.2 M hydrochloric acid and 0.2 M sodium hydroxide solutions.

A 0.01 M sodium tetraborate solution was used as a carrier solution for the FI system.

A bromocresol green solution (10^{-4} M) was prepared by dissolving 0.0698 g bromocresol green powder (acid form) in 2 ml of 0.1 M sodium hydroxide, diluting to 1000 ml with an acid phthalate buffer of desired pH and filtering before use.

2.4. Absorption spectrum study

Absorption spectra of an extract were investigated. Extraction was carried out by mixing 5 ml

of a diphenhydramine hydrochloride standard solution (a concentration range of 3–15 μg ml^{-1}) with 5 ml of bromocresol green solution (pH 3) and shaking with 10 ml of chloroform in a 20 ml stoppered vial for 1 min.

Aiming for a simple FI manifold, handling with only aqueous layer of the extraction was preferred rather than dealing with an organic solvent. Thus, the spectra of the excess bromocresol green in aqueous layer (pH 3) and those after adjusting to be alkaline were recorded.

2.5. Manifold for FI-spectrophotometry

A single-line manifold (Fig. 1) was employed. A sample was injected through a six-port injection valve with a sample loop of 100 μl (Upchurch, USA) into a stream of carrier solution pumped by a peristaltic pump (Eyela, Japan) to an 80 μl flow through cell (1 cm path length, Hellma, Germany) sitting in a spectrophotometer (Spectronic 21, USA) connected to a chart recorder (Hitachi, model 056-1002, Japan). Tygon tubings (1 mm ID) and flexible pump tubes were employed.

Standard/sample solutions were extracted off-line by following the procedure for the absorption spectrum study described earlier. An aqueous layer was then injected into the system.

2.6. Analysis of samples

A weighed quantity of a sample was dissolved with deionized water, shaken for 15 min and adjusted to a volume with water to obtain a solution having a concentration in the range of a calibration graph. For a sample with high pH (pH > 7), an acid phthalate buffer pH 3 was used instead of water for dilution. An aliquot of the sample solution (5 ml) was transferred to a 20 ml stoppered vial, added with 5 ml of bromocresol green solution (pH 3) and 5 ml of chloroform, extracted by shaking for 1 min, and then an aqueous layer was taken for centrifuging for complete separation and clarification before being injected into the FI system. Replicate injections of the aqueous solution were made. The samples were also analyzed in triplicate by HPLC for referee purpose.

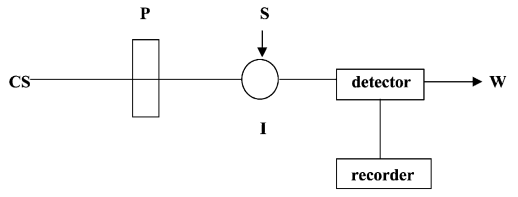


Fig. 1. FI manifold for diphenhydramine hydrochloride determination; CS, carrier solution; P, peristaltic pump; I, six-port injection valve; S, standard/sample; W, waste.

3. Results and discussion

3.1. Absorption spectra

The spectra of an excess bromocresol green in aqueous layer (pH 3.0) after extraction (Section 2.4) were recorded from 220 to 600 nm and from 220 to 800 nm for the one after being adjusted to be alkaline (pH 10) as shown in Fig. 2 and Fig. 3 respectively. The maximum absorption wavelengths of those exhibited at 435 and 615 nm, respectively. The latter was chosen for analytical purposes since it provides better molar absorptivity.

3.2. Optimization

A series of diphenhydramine hydrochloride standard solutions (3–21 μg ml^{-1}) and 1×10^{-4} M of bromocresol green solution were used. The chemical parameters: carrier solution (0.005–0.1 M NaOH and 0.002–0.1 M borax); and pH of reagent (prepared in an acid phthalate buffer of pH 2–5) and FI parameters: mixing coil length (between the injection valve and the detector, 0–100 cm); and sample volume (60–200 μl) were evaluated by injecting the aqueous layer containing excess bromocresol green (obtained from the extraction) into the manifold. Preliminary conditions were as following: carrier flow rate, 5.0 ml min^{-1}; sample injection volume, 100 μl; mixing coil length, 50 cm; and wavelength of measurement, 610 nm.

According to the preliminary study, the simple FI system (Fig. 1) was carried out for determining

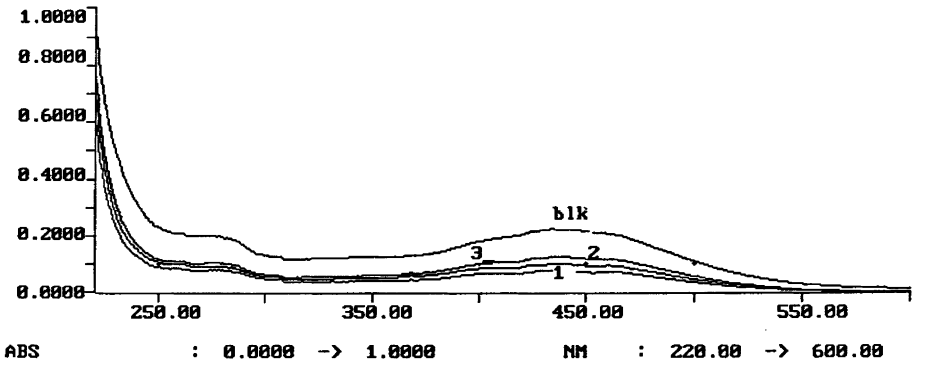


Fig. 2. Absorption spectra of the excess bromocresol green in aqueous layer (pH 3) after extraction. Diphenhydramine hydrochloride concentrations: [blk = blank], 0; [1], 15; [2], 9; and [3], 6 μg ml^{-1}. Bromocresol green concentration: 1×10^{-4} M.

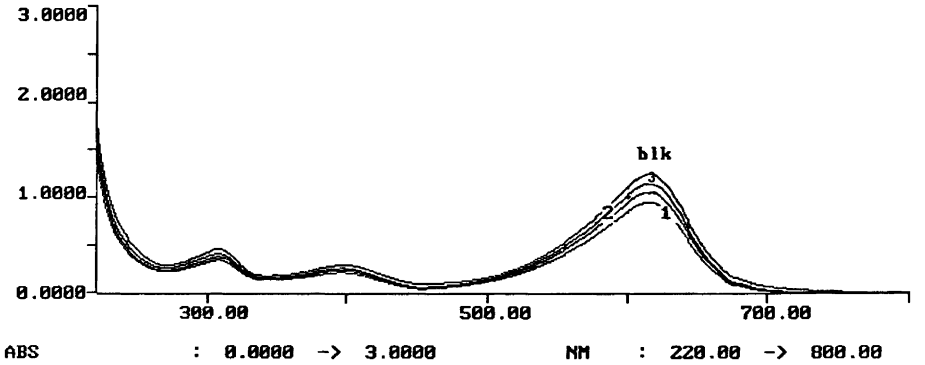


Fig. 3. Absorption spectra of the excess bromocresol green in aqueous layer after being adjusted to be alkaline (pH 10). Diphenhydramine hydrochloride concentrations: [blk = blank], 0; [1], 15; [2], 9; and [3], 6 μg ml^{-1}. Bromocresol green concentration: 1×10^{-4} M.

the excess bromocresol green in aqueous layer by injecting the dye into an alkaline (pH 10) carrier stream and continuously monitored at 610 nm. A 0.01 M borax was chosen as the carrier solution due to providing better reproducible responses, peak height and sensitivity. It was found similarly to the previous findings [12] that the higher pH of reagent decreased the extraction efficiency of ion-pair compound, extraction should be performed by using the reagent of a lower pH (pH 3). As expected, the longer the mixing coil, the lower signals were obtained due to the higher dispersion. A manifold without a mixing coil resulted the highest signals. For the effect of injection volume: 60, 100, 150 and 200 μl, calibrations of $y = -5.02x + 142.9$; $y = -5.75x + 191.5$; $y = -8.00x + 250.8$ and $y = -7.30x + 251.6$ were obtained, respectively. The volume of 100 μl was considered for a better sample throughput.

Proposed conditions are listed in Table 1.

Table 1
Conditions for the determination diphenhydramine hydrochloride in some pharmaceutical preparations by spectrophotometry with FI

Carrier solution	0.01 M borax
Flow rate of carrier solution	5.0 ml min^{-1}
Sample volume	100 μl
Mixing coil length	0 cm
Flow through cell volume	80 μl
Measurement wavelength	610 nm
Sensitivity of recorder	1 V
Chart speed of recorder	5 mm min^{-1}

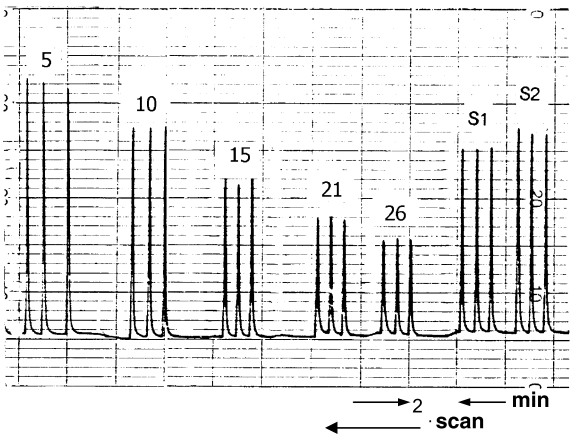


Fig. 4. FI recording obtained for different diphenhydramine hydrochloride standard solutions (5–21 μg ml^{-1}) (see text).

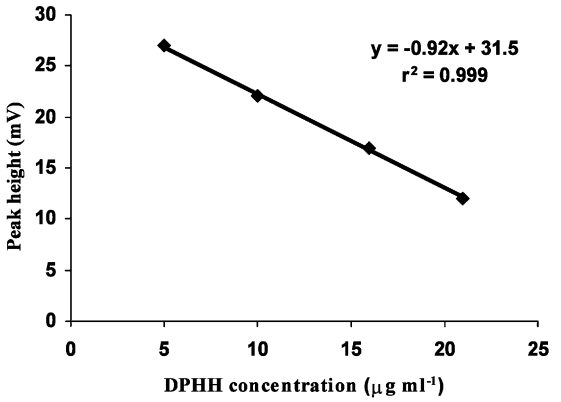
3.3. Analytical characteristics

FI-gram of the excess bromocresol green in the aqueous layer, related to the amount of diphenhydramine hydrochloride is shown in Fig. 4.

According to the diminution of bromocresol green relating to the amount of diphenhydramine hydrochloride, the bromocresol green concentration used should provide the greatest color contrast and sensitivity. Series ranging in concentrations of 5–188 μg ml^{-1} standard solutions and $1–5 \times 10^{-4}$ M of bromocresol green solutions were investigated. Calibration plots of diphenhydramine hydrochloride concentration (μg ml^{-1}) versus peak height (mV) were found to be linear in the range of 5–21 μg ml^{-1} using 1×10^{-4} M of bromocresol green solution ($y = -0.92x + 31.5$, $r^2 = 0.999$) (Fig. 5) and of 75–188 μg ml^{-1} using 5×10^{-4} M of bromocresol green solution ($y = -5.89x + 1207.2$, $r^2 = 0.991$) (Fig. 6) with the detection limits (3σ) [22] of 1 and 15 μg ml^{-1}, respectively.

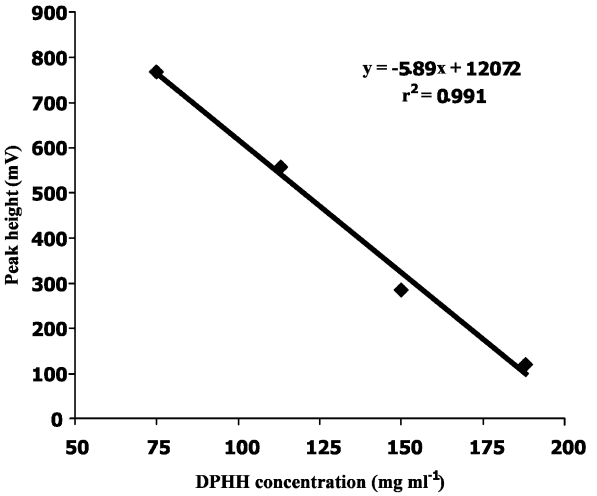
Eleven injections of an extracted standard solution, containing 107 μg ml^{-1} diphenhydramine hydrochloride were performed in order to evaluate the precision of FIA system and injection rate; the results obtained were 1.6% RSD and 100 injections h^{-1}, whereas the precision of a complete procedure and efficiency of the extraction were investigated from 7 replicate determinations (triplicate injections each) of the standard solution containing 107 μg ml^{-1} diphenhydramine hydrochloride. The RSD was also found to be 1.6% and the efficiency of extraction was 93%.

The tolerance of the method to foreign compounds commonly presenting in pharmaceutical preparations was investigated by using a solution similar to those used for the precision studies and adding various concentrations of the compounds commonly found in some pharmaceutical preparations: capsule; syrup and lotion. The results obtained (Table 2) indicate that the amount of the foreign compounds commonly presented together with diphenhydramine hydrochloride in preparations of capsule or syrup containing single tertiary



DPHH (μg ml^{-1})	Peak height (mV)
5	27
10	22
16	17
21	12

Fig. 5. Calibration graph; means of triplicate injections (5–21 μg ml^{-1} of diphenhydramine hydrochloride, 1×10^{-4} M of bromocresol green solution).



DPHH (μg ml^{-1})	Peak height (mV)
75	768
113	557
150	285
188	120

Fig. 6. Calibration graph; means of triplicate injections (75–188 μg ml^{-1} of diphenhydramine hydrochloride, 5×10^{-4} M of bromocresol green solution).

alkylamine drug would not interfere in the determination. Dextromethorphan hydrobromide and bromhexine hydrochloride do interfere seriously. The proposed procedure should only be appropriate for an assay of a preparation containing diphenhydramine hydrochloride as a single tertiary alkylamine. Some compounds such as sodium citrate could have effected on pH, however, it could be solved by adjusting the solution with acid phthalate buffer pH 3 before extraction.

3.4. Analysis of samples

Application of the proposed FI spectrophotometric procedure to assay diphenhydramine hydrochloride in some pharmaceutical preparations (capsule and syrup samples which contain single tertiary alkylamine) was demonstrated. A sample was prepared to obtain solutions having concentrations with appropriate dilution of the sample, in the ranges of calibrations of 5–21 μg ml^{-1} or 75–188 μg ml^{-1}. The results were compared to the analysis results with HPLC, as a referee method [19] (Table 3). Evaluation by *t*-test [23] at 95% confidence level indicates that there is no significant difference in the results obtained by the proposed FIA and HPLC procedures. It should be noted that diphenhydramine hydrochloride content in a lotion sample (Caladryl®: diphenhydramine hydrochloride, 1% w/w; calamine, 8% w/w; camphor, 0.1% w/w; alcohol, 2% w/w, pH 7.5) was also determined. The results obtained for

Table 2
Effects the interfering compounds, commonly found in pharmaceutical preparations on the determination of 107 μg ml^{-1} of diphenhydramine hydrochloride; means of 11 replicate injections

Substances [a][a]	Amount ratio (by weight)	%Relative error
Sucrose [170]	370 000	0.8
Lactose [4]	179	3.5
Ammonium chloride [10]	42	0.0
Citric acid [3.2]	13	2.5
Sodium citrate[b] [4]	8	3.6
Camphor [0.1]	38	1.7
Dextromethorphan·HBr [0.75]	0.3	35.4
	0.6	67.1
Bromhexine·HCl [1]	0.05	11.8
	0.27	34.2
Glyceryl guaiacolate [12.5]	21	3.5

[a] Amount ratio by weight may be found in pharmaceutical preparations.
[b] Adjusted with acid phthalate buffer pH 3.

Table 3
Determination of diphenhydramine hydrochloride in some pharmaceutical preparations

Preparation	Procedure[a]			
	FIA[b]		HPLC[c]	
	Amount found	%la	Amount found	%la
Capsule[d] (mg^{-1} cap)	25.4 ± 1.0	101.6	25.0 ± 0.5	100.0
Syrup A[e] (mg 5 ml^{-1})	12.8 ± 0.1	102.8	12.6 ± 0.1	100.8
Syrup B[f] (mg 5 ml^{-1})	11.5 ± 0.6	92.0	11.6 ± 0.6	93.2

[a] Mean of 3 determinations.
[b] FIA procedure: for sample preparation see text and using calibration range of 5–21 μg ml^{-1}.
[c] USP 24 [19].
[d] Benadryl® capsule: diphenhydramine hydrochloride 25.0 mg cap.
[e] Benadryl® cough syrup: diphenhydramine hydrochloride, 12.5 mg 5 ml; ammonium chloride; 125.0 mg 5 ml; sodium citrate, 50.0 mg 5 ml; menthol, 1.0 mg 5 ml; alcohol, 5% v/v.
[f] Cotussin® cough syrup: diphenhydramine hydrochloride, 12.5 mg 5 ml; ammonium chloride, 125.0 mg 5 ml.

the sample diluted with water and acid phthalate buffer pH 3 were found to be 66.7% la. and 83.0% la., respectively. This could be that dilution of a sample of high pH (pH > 7) with the buffer of pH 3 to yield lower pH before extraction could increase efficiency of extraction and hence yields the better results. Further investigation should be attempted for such a type of sample.

4. Conclusion

Spectrophotometry with simple FI is proposed to the determination of diphenhydramine hydrochloride in some pharmaceutical preparations, which contain single tertiary alkylamine. Application of the proposed procedure has been demonstrated for an assay for capsule and syrup samples. The procedure is simple and economics and could be an alternative for places where HPLC equipment is not available.

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