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Journal of Pharmaceutical and Biomedical Analysis
21 (1999) 709–714

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Flow-injection extraction-spectrophotometric method for the determination of chlorhexidine in pharmaceutical preparations

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Received 2 December 1998; received in revised form 23 March 1999; accepted 5 May 1999

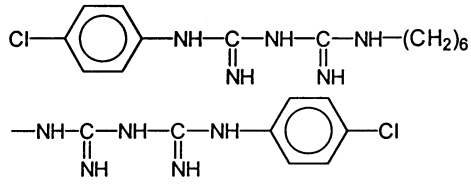
Abstract

The spectrophotometric determination of trace amounts of chlorhexidine was carried out by liquid-liquid extraction using bromophenol blue with a flow system. The determination of chlorhexidine in the range of 1×10^{-4} to 1×10^{-5} M was possible with a sampling frequency of 40 samples per hour. The method was satisfactorily applied to the determination of chlorhexidine in pharmaceutical preparations. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Chlorhexidine; Bromophenol blue; Flow-injection; Extraction

1. Introduction

Chlorhexidine, a bactericidal drug widely used as an antiseptic, is a member of the biguanide family [1,1′-hexamethylene bis(5-(*p*-chlorophenyl) guanide]. On its own or in combination with other active principles, it is incorporated in pharmaceutical preparations of various degrees of complexity.



Various analytical techniques have been used for its quantitative analysis. Titration in non-aqueous medium is suitable for the determination of relatively large amounts of the drug [1]. Other methods include spectrophotometry based on its reaction with different dyes [2–4], polarography, differential-pulse adsorptive stripping voltammetry [5–7] and several chromatographic techniques, especially HPLC [8–17].

There is a constant search for simple, reliable, automated and semiautomated methods for the rapid quantification of substances of therapeutic interest in pharmaceutical samples and biological fluids. However, only three flow-injection methods for the determination of chlorhexidine have

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PII: S0731-7085(99)00207-1

been described in the literature, all of them based on the formation of insoluble complexes between chlorhexidine and thymol blue [18], copper [19], or bromocresol green [20]. The detection system used was turbidimetry for thymol blue, absorption atomic spectrometry for copper(II) and spectrophotometry for bromocresol green, using Triton X-100 micelles to dissolve the complex. The calibration graphs were very narrow (only six times in the most favourable case) and the sensitivity was not high. Flow-injection (FI) in association with extraction in organic solvents has made it possible to automate and speed up the handling of reagents in routine analysis with good selectivity and sensitivity.

The purpose of this work was to investigate the formation and extraction behaviour of ion-pairs of chlorhexidine with acid dyes in order to develop useful automatic spectrophotometric methods. The results showed that bromophenol blue and chloroform were the most effective dye and extractant, respectively, for use in unsegmented flow configuration using a continuous extraction system. This system overcame the complexity of the manual extraction methods and avoided the troubles and hazards involved in handling toxic organic solvents. Flow injection minimizes the above shortcomings since the organic solvents are kept in closed vessels. The proposed automatic method was applied to the determination of chlorhexidine in pharmaceutical preparations.

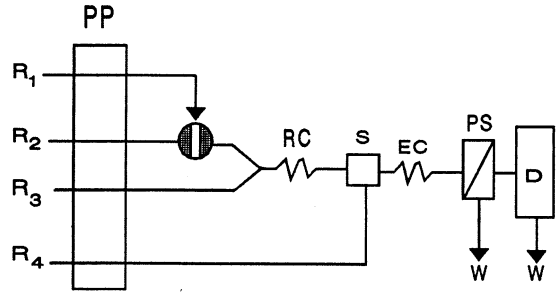

Fig. 1. Manifold for determination of chlorhexidine. D, detector; EC, extraction coil (300 cm × 0.5 mm i.d.); PP, peristaltic pump; PS, phase separator; R_1, sample; R_2, buffer (pH 2); R_3, bromophenol blue; R_4, chloroform; RC, reaction coil (150 cm × 0.5 mm i.d.); S, segmentor; W, waste.

2. Materials and methods

2.1. Reagents

Chlorhexidine was obtained from Sigma (St. Louis, MO, USA) and used as received. A standard 1.0×10^{-3} M solution was prepared by dissolving the drug in distilled water; this solution remained stable if kept refrigerated. Working solutions of lower concentrations were freshly prepared by appropriate dilution of the standard solution.

Stock solutions (1×10^{-3} M) of bromophenol blue, bromothymol blue, orange IV and methyl orange were prepared by dissolving the required amount of the dye (Sigma) in distilled water. Solutions of lower concentration were prepared by dilution of the stock solution with distilled water.

All solvents used (chloroform, 1,2-dichloroethane, methyl isobutyl ketone and ethyl acetate) were of analytical reagent grade.

2.2. Apparatus

A Perkin-Elmer (Norwalk, CA, USA) 550 SE spectrophotometer was used to record the spectra, and a Pye-Unicam (Cambridge, UK) 8625 spectrophotometer was used as the detector in the flow system. A Gilson (Villiers le Bell, France) Minipuls HP4 peristaltic pump fitted with Tygon and Acidflex pump tubes and an Omnifit (Cambridge, UK) injection valve were also used.

2.3. Manifold

The configuration of the flow-injection manifold used is depicted in Fig. 1, with the optimum conditions as stated. Chloroacetate buffer of pH 2.0 and bromophenol blue solutions were pumped through Tygon tubes and chloroform was pumped through the Acidflex tube. The sample (200 μl) was introduced into the buffer stream by means of an Omnifit rotary valve, to which a volume control loop was attached. All connecting tubing was made of poly(tetrafluoroethylene) (PTFE). A T-segmenter, in which the aqueous phase flows straight and the organic phase at

Table 1
Extraction of chlorhexidine-dye ion-pairs[a]

Dye	A_{blank}	$A_{ion\text{-}pair}$
Bromophenol blue	0.006	0.522
Bromothymol blue	0.100	0.546
Methyl orange	0.026	0.510
Orange IV	0.050	0.063

[a] Chlorhexidine concentration: 2×10^{-5} M; dye concentration: 1×10^{-4} M. A_{blank} values correspond to the absorbances of organic extracts of the samples containing all reagents in the absence of chlorhexidine.

right-angles, was used to mix both phases. The extraction coil was 300 cm long. The phase separator was constructed from solid PTFE which had an inlet and two outlets (bore 0.5 mm i.d.). The three-threaded hole accepted the standard polypropylene end pieces. During operation the two blocks were pressed together with the aid of two stainless steal clamps. A porous PTFE membrane with 1.0-μm pore size (Fluoropore, Milipore Ibérica, Madrid, Spain), permeable to chloroform but impermeable to the aqueous solution, was sandwiched between the two blocks. A grid placed between the membrane and the inner non-grooved surface of the block prevented the membrane from collapsing into the recipient chamber, the volume of which was only 20 μl. A grooved phase separator with PTFE membrane (1.0-μm pore size) was used. The absorbance of the organic phase was measured at 422 nm with a spectrophotometer equipped with a Hellma (Jamaica, NY, USA) 178.012 QS flow cell (18-μl inner volume and 10-mm light-path length) and was recorded with a Linseis (Selb, Germany) 6215 recorder.

2.4. Sample preparations

The tablets containing chlorhexidine were finely powdered and weighed. An amount of this powder, equivalent to ~5 mg of chlorhexidine, was accurately weighed and shaken with 20 ml of distilled water and 10 ml of 5 M hydrochloric acid in a water-bath at 50°C for 10 min. After cooling, the solution was filtered and the residue was washed several times with 0.1 M hydrochloric acid. The combined filtrate and washings was adjusted to a pH of ~5–6 with 5 M sodium hydroxide and diluted with distilled water to 100 ml in a calibrated flask to obtain a solution of 50 μg ml^{-1}.

For the determination of chlorhexidine in solutions, a quantity equivalent to 5 mg of the drug was transferred to a 100-ml volumetric flask and diluted to the mark with distilled water.

Toothpaste (5 g) was boiled with 250 ml of 2 M HCl. After cooling, the solution was filtered, adjusted to pH 5–6 with 5 M sodium hydroxide and concentrated with a rotary evaporator to ~20 ml. The concentrate was transferred to a 25-ml calibration flask and diluted to volume with distilled water.

3. Results and discussion

Chlorhexidine can be transferred from the aqueous phase into the organic phase as an ion-pair formed with the anionic form of the acid dyes. The extraction equilibria can be represented as follows:

$$CH^{+}_{(aq)} + D^{-}_{(aq)} \Leftrightarrow CH^{+}D^{-}_{(aq)} \Leftrightarrow CH^{+}D^{-}_{(org)}$$

where CH^{+} and D^{-} represent the protonated chlorhexidine and the anion of the dye, respectively, and the subscripts aq and org refer to the aqueous and organic phases, respectively.

The dyes studied for chlorhexidine ion-pair formation were bromophenol blue, orange IV, methyl orange and bromothymol blue. Of the dyes tested, bromophenol blue showed the greatest ion-pair extraction efficiency with the smallest reagent blank extraction (Table 1).

The effect of the extracting solvent used was also examined since the polarity of the solvent affects both extraction efficiency and absorbance. The results using bromophenol blue are shown in Table 2. In this study, bromophenol blue and chloroform were selected because of the high sensitivity, very low absorbance of the reagents blank and the shortest time to reach the equilibrium between both phases.

Table 2
Effect of the extracting solvent on absorbance of the chlorhexidine-bromophenol blue ion-pair[a]

Solvent	A_{blank}	$A_{ion\text{-}pair}$
Chloroform	0.006	0.522
1,2-Dichloroethane	0.007	0.500
Methyl isobutyl ketone	0.270	0.837
Ethyl acetate	0.215	0.890

[a] Chlorhexidine concentration: 2×10^{-5} M; bromophenol blue concentration: 1×10^{-4} M.

3.1. Characteristics of the chlorhexidine-bromophenol blue ion-pair

Bromophenol blue and the ion-pair have identical spectra and so they must be separated if the ion-pair is to be quantified.

The effect of pH on the formation and extraction of the ion-pair was studied using universal buffer Britton-Robinson solutions over the range 1.5–6.0. The absorbance of the organic extract was maximum at pH 2 (Fig. 2).

The composition of the ion-pair was established by Job's method of continuous variations [21] and by the molar ratio method [22] using both variable dye concentrations and variable chlorhexidine concentrations. The results obtained with these methods showed a molar ratio between dye and chlorhexidine of 3:1. The extraction constant for the above equilibrium was $\log K_{ex} = 16.49 \pm 0.25$.

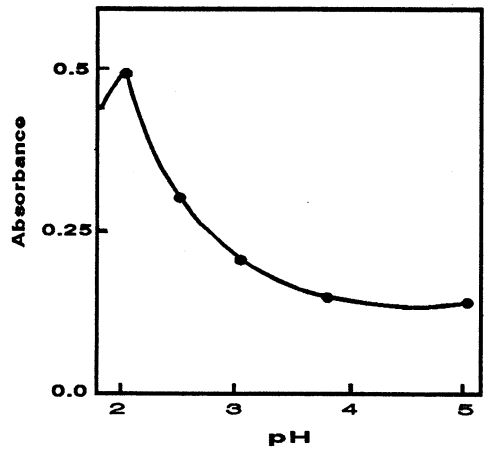

Fig. 2. Influence of pH on the extraction of ion-pair. [Chlorhexidine] = 5×10^{-5} M. [Bromophenol blue] = 3×10^{-4} M.

Shaking times ranging from 0.5 to 5 min did not produce any change in the absorbance, suggesting that equilibrium between the two phases in the extraction of the ion-pair can be attained rapidly. Reproducible absorbance readings were always obtained after a single extraction. The overall extraction efficiency was 96.8%.

3.2. Flow-injection determination of chlorhexidine

The flow manifold (Fig. 1) for the automation of the proposed method was arranged so as to consider the essential features of chlorhexidine-bromophenol blue ion-pair. The universal buffer Britton-Robinson was replaced by the monochloroacetate buffer in order to use a simpler buffer with a high buffering capacity at pH 2.0.

3.2.1. Influence of manifold parameters

The optimization of the manifold parameters with respect to sensitivity, peak resolution, phase separation efficiency and rapidity of the analysis was carried out using the results obtained from the batch studies. The carrier was a monochloroacetate buffer of pH 2 (0.1 M) and the reagent stream was an 3×10^{-4} M bromophenol blue solution.

The flow-rate of the aqueous and organic streams were varied in order to obtain the maximum concentration coefficient without significantly decreasing the sample throughput. The optimization of flow-rate resulted in the adoption of 1.3 (0.65 for each channel) and 1.3 ml min^{-1} for the aqueous and organic streams, respectively (Fig. 3).

The tube length between the valve and segmenter (ion-pair reaction coil) was varied from 20 to 150 cm (0.5 mm i.d.) A reaction coil of 100 cm was sufficient to obtain the maximum absorbance because the ion-pair forms rapidly.

The influence of the extraction coil length was also examined. The peak height increased as the extraction coil increased in length up to 270 cm,

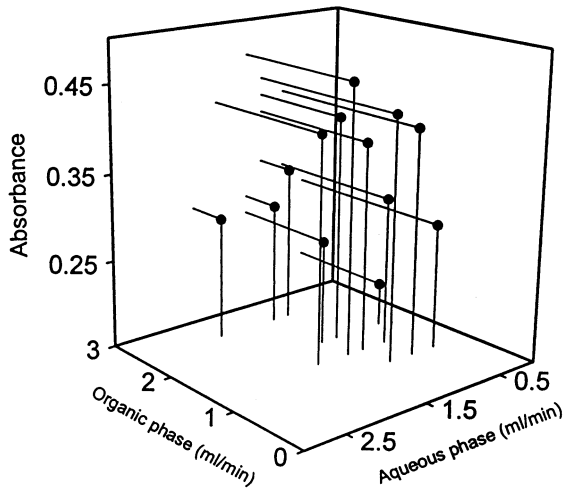

Fig. 3. Influence of aqueous and organic stream flow rates on the extraction efficiency of the chlorhexidine-bromophenol blue ion-pair.

above which the signal remained virtually constant. An extraction coil length of 300 cm (0.5 mm i.d.) was selected.

The volume of sample injected was varied from 35 to 250 μl by changing the length of the sample loop in the injection valve. The peak height increased with increasing sample size up to 200 μl, above which it remained virtually constant. The volume to be injected was selected as 200 μl.

Table 3
Tolerance to different species in the determination of chlorhexidine[a]

Species added	Maximum tolerable mole ratio
Sucrose, glycerine, urea, sorbitol, ascorbic acid, saccharine, sodium fluoride	100[b]
Citric acid, benzoic acid, glycerol, benzyl alcohol, methyl *p*-hydroxybenzoate, menthol, benzocaine, propyl *p*-hydroxybenzoate, sodium cyclamate, tyrothricin	50

[a] Chlorhexidine = 5×10^{-5} M.
[b] Maximum ratio tested.

3.2.2. Effect of the reagent concentration

Using a 3×10^{-4} M bromophenol blue solution as reagent stream, the pH of the buffer solution (carrier) was varied between 1.5 and 4.0. The peak height was maximum and constant from pH 1.7 to 2.2, and decreased outside this range. Therefore, a 0.1-M monocloroacetate buffer of pH 2.0 was used as carrier. With the carrier stream buffered at pH 2.0, the concentration of bromophenol blue was varied between 1×10^{-4} and 8×10^{-4} M. Peak height increased with increasing concentrations of the dye solution stream up to 2×10^{-4} M, but levelled off at higher concentrations. The concentration adopted in the procedure was 3×10^{-4} M.

3.2.3. Calibration graph and reproducibility

The effect of the concentration of chlorhexidine on the absorbance was studied by measuring the peak height when 200 μl of chlorhexidine hydrochloride solution at different concentrations were injected. The calibration graph was found to be linear between 1.0×10^{-6} and 1.0×10^{-4} M (57 840–5784 μg/ml), and the regression equation obtained was:

$$A = (2.95 \times 10^{-2} \pm 7.4 \times 10^{-3}) + (4686.67 \pm 1.197)\ C; \quad (r = 0.9987)$$

where C is the concentration of chlorhexidine in molar, A the absorbance and r the correlation coefficient. The relative standard deviation of ten injections of each solution containing 23.13 and 34.70 μg ml^{-1} of chlorhexidine were 0.96 and 0.81%, respectively. The detection limit, calculated to IUPAC recommendations [23], was 6.8×10^{-6} M. The sampling rate was 40 samples per hour.

The reproducibility of the method was studied by analysing, on 5 different days, ten identical solutions of chlorhexidine (8.0×10^{-5} M). Every day three injections of each solution were made; the relative standard deviation for the peak height was 1.32%.

3.2.4. Interference studies

In order to apply the proposed method to the analysis of pharmaceutical dosage forms, the influence of commonly used excipients and additives

Table 4
Determination of chlorhexidine in pharmaceutical preparations

Preparation	Supplier	Amount found[a] (mg)
Perio. aid (1.2 mg per ml)	Dentaid	1.18 ± 0.01
Cariax gingival (1.2 mg per ml)	Kim	1.21 ± 0.01
Eludril (1 mg per ml)	Pierre Fabre	0.98 ± 0.02
Cristalmina (10 mg per ml)	Bama-Geve	10.16 ± 0.02
Drill (3 mg per tablet)	Pierre Fabre	2.92 ± 0.01
Faringesic (5 mg per tablet)	Diafarm	4.93 ± 0.03
Hibitane (5 mg per tablet)	Smithkline, Beecham	5.06 ± 0.05
Bucometasana (5 mg per tablet)	Solvay Pharma	5.06 ± 0.02
Elgydium (tooth paste) (0.040 mg per g)	Pierre Fabre	0.0392 ± 0.0002

[a] Mean of four determinations $\pm$ S.D.

was studied by preparing solutions containing 5×10^{-5} M of chlorhexidine and different amounts of the foreign compounds. Tolerance was defined as the amount of foreign substance inducing errors lower than 3% in the determination of the analyte. Table 3 shows the results obtained.

3.2.5. Analysis of pharmaceutical preparations

In order to establish the validity of the proposed method, several pharmaceutical preparations were analysed. Interferences from the matrix were not a problem. The data in Table 4 show that the assay results were in good agreement with the labelled contents The recoveries obtained for chlorhexidine to each pharmaceutical formulations ranged from 97.2 to 102.6%.

4. Conclusions

The above results clearly indicate that chlorexidine can successfully be determined by extraction as ion-pair with bromophenol blue in a flow-injection assembly. The method has the general advantages of FI, namely instrumental simplicity, high sampling rate, economy in use of reagents and decreased exposure to organic solvent vapours. The proposed method is useful for determining chlorhexidine in pharmaceutical dosage forms.

Acknowledgements

The authors are thankful for the financial support given by Dirección General de Investigación Científica y Técnica (Proyect PB96-1101) and Fundación Séneca (PB-13/FS/97).

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