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Flow-injection extraction-spectrophotometric determination of bromhexine with orange IV

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Abstract

An automatic flow-injection photometric method for the determination of bromhexine is proposed. The drug was determined by formation of an ion-pair with orange IV, extraction into 1,2-dichloroethane and measurement of the absorbance at 412 nm of the organic phase. A linear calibration graph was obtained at concentrations of 5×10^{-6}–1.6×10^{-4} M of bromhexine. Up to 40 samples h^{-1} can be processed with an RSD of 0.32–0.88%. The method was applied to the determination of bromhexine in blood serum and a pharmaceutical preparation.

Keywords: Bromhexine; Flow-injection method; Ion-pair extraction; Orange IV; Photometry

1. Introduction

Bromhexine (2-amino-3,5-dibromo-*N*-cyclohexyl-*N*-methylbenzenemethanamine) is a highly substituted aniline derivative which has been shown to decrease the viscosity and increase the volume of mucus. At least part of its activity has been attributed to its ability to fragment mucopolysaccharide fibres. Bromhexine hydrochloride has been formulated alone and with other drugs, and is available commercially as drops, tablets and granules for administration as a bronchial mucolytic agent.

Many methods have been used for the determination of bromhexine, including gas/liquid chromatography in conjunction with electron capture, mass spectrometry or flame-ionization [1–4] and high-performance liquid chromatography with UV-detection [5,6]. The diazotization of bromhexine has been used for its determination in two ways. One involves potentiometric titration with sodium nitrite [7] and the other involves spectrophotometric measurement of the compound formed by the coupling of the diazotized bromhexine derivative with *N*-(1-naphthyl)ethylenediamine (NED), resorcinol or phloroglucinol [8–10]. Methods based upon the formation of ion-pairs and their extraction in organic solvents using bromocresol purple [11] and various metal-containing reagents [12–15] have been used to determine bromhexine by molecular and atomic absorption spectrometry (AAS).

Bromhexine has also been determined automatically by applying kinetic methodology using the stopped-flow mixing technique to the coupling of the diazotized bromhexine derivative with NED [16]. A continuous liquid–liquid extractor coupled on-line to an atomic absorption spectrometer has also been used for the indirect AAS determination of bromhexine using the inorganic complexes BiI_4^-, $Co(SCN)_4^{2-}$ and Reinecke's salt to form and extract various ion-pairs with bromhexine [14,15].

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The purpose of this work was to investigate systematically the formation and extraction behaviour of ion-pairs of bromhexine with acid dyes in order to develop useful automatic photometric methods. The proposed automatic method has been applied to the determination of bromhexine in blood serum and pharmaceutical preparations.

2. Experimental

2.1. Reagents

Bromhexine hydrochloride 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.

A 1.0×10^{-3} M orange IV (4-(*p*-anilinophenyl-azo)benzenesulphonic acid sodium salt) stock solution was prepared by dissolving the required amount of the dye (Sigma) in water. Solutions of lower concentration were prepared by dilution of the stock solution with distilled water.

2.2. Apparatus

A Perkin-Elmer (Norwalk, CA, USA) 550 SE spectrophotometer was used for recording 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 is depicted in Fig. 1 with the optimum conditions as stated. Chloroacetate buffer and orange IV solutions were pumped through Tygon tubes and 1,2-dichloroethane was pumped through the Acidflex tube. The sample (60 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 right-angles, was used for mixing both phases. The length of the extraction coil was 100 cm. 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 pins. A porous PTFE membrane (1.0-μm pore size), permeable to chloroform but impermeable to the aqueous solution, was sandwiched between the two blocks. A grid placed between the membrane and the inside non-grooved surface of the block prevented the membrane from collapsing into the recipient chamber, the volume of which was only 20 μl. The absorbance of the organic phase was measured at 412 nm with a spectrophotometer equipped with a Hellma (Jamaica, NY, USA) 178.012 OS flow cell (18 μl inner volume and 10-mm light-path length) and was recorded with a Linseis (Selb, Germany) 6215 recorder.

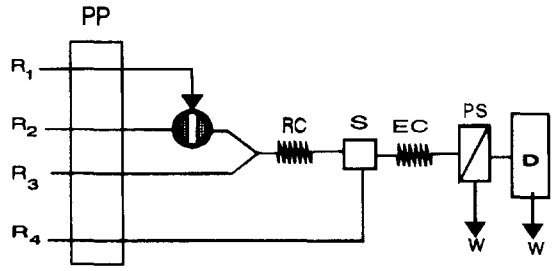

Fig. 1. Manifold for determination of bromhexine: PP = peristaltic pump; R_1 = sample; R_2 = chloroacetate buffer (pH 3); R_3 = orange IV solution; R_4 = 1,2-dichloroethane; RC = reaction coil (30 cm × 0.5 mm i.d.); S = segmentor; PS = phase separator; EC = extraction coil (100 cm × 0.5 mm i.d.); W = waste; D = detector.

Table 1
Effect of the extracting solvent on the absorbance of the ion-pair with orange IV

Solvent	Relative absorbance (%)	
	Ion-pair	Reagent blank
1,2-Dichloroethane	100	0
Chloroform	97	0
Tetrachloroethane	16	12
Ethyl acetate	53	14
Isopentyl acetate	42	34
Isobutyl methyl ketone	86	48

3. Results and discussion

The formation of ion-pairs between bromhexine and ionogens can be used to isolate the drug from an aqueous phase. When the ionogen selected is a chromophore, an extraction-photometric method can be developed.

Bromhexine can be transferred from the aqueous phase into the organic phase as an ion-pair formed with the anionic form of the acid dye. The following steps take place:

$$BHX^{+}_{(aq)} + D^{-}_{(aq)} \rightleftharpoons BHX^{+}D^{-}_{(aq)} \rightleftharpoons BHX^{+}D^{-}_{(org)}$$

where BHX^{+} and D^{-} represent the protonated bromhexine and the anion of the dye respectively.

The dyes studied for bromhexine ion-pair formation were eriochrome cyanine R, erythrosine B, rose bengal, chromazurol S, bromothymol blue, methylthymol blue, methyl orange and orange IV. Of the dyes tested, orange IV showed the greatest ion-pair extraction efficiency with the smallest reagent blank extraction.

The effect of the extracting solvent was also examined. The polarity of the solvent affects both the extraction efficiency and the absorbance. The results using orange IV are shown in Table 1, in which the response using 1,2-dichloroethane was normalized as 100. In this study, 1,2-dichloroethane was preferred to chloroform because of its lower volatility.

3.1. Characteristics of the bromhexine-orange IV ion-pair

Orange IV and the ion-pair $BHX^{+}D^{-}$ 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 solutions over the pH range 2.0–6.0. The absorbance of the organic extract was maximum and constant in the pH range 2.3–3.2 (Fig. 2).

The composition of the ion-pair was established by Job's method of continuous variations, and by the molar ratio method using both variable dye concentration and variable bromhexine concentration. The results obtained with these methods showed that the composition of the ion-pair was equimolar (1:1). The extraction constant for the equilibrium (1) was $\log K_{ex} = 4.71 \pm 0.29$.

Shaking times of 0.5–5 min did not produce any change in the absorbance, suggesting that

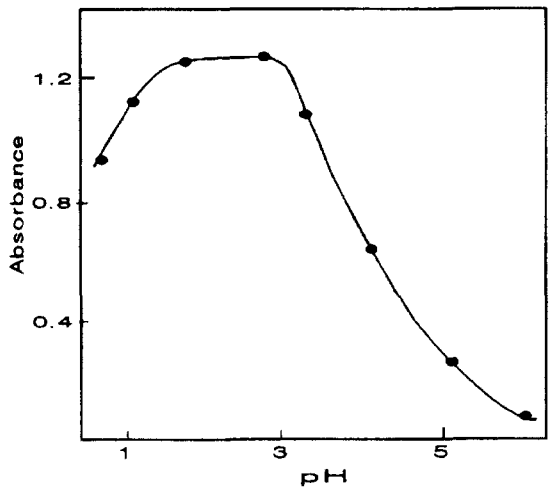

Fig. 2. Influence of pH on the extraction of the ion-pair: [bromhexine] = 1×10^{-4} M; [orange IV] = 4×10^{-4} M.

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 96.3%.

3.2. Flow-injection determination of bromhexine

The flow manifold (Fig. 1) for the automation of the proposed method was arranged so as to consider the essential features of determining the bromhexine-orange IV ion-pair.

Flow-injection variables

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 chloroacetate buffer pH 3 (0.2 M) and the reagent stream was an orange IV solution (5×10^{-4} M). The concentration of the sample solution was 1.0×10^{-4} M.

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 1.8 (0.9 for each channel) ml min^{-1} and 1.3 ml min^{-1} for the aqueous and organic streams, respectively.

The tube length between the valve and segmenter (ion-pair reaction coil) was varied from 20 to 60 cm (0.5 mm i.d.) A reaction coil of 30 cm length was sufficient to obtain the maximum absorbance because the ion-pair is formed rapidly.

The influence of the extraction coil length was also examined. The peak height increased as the extraction coil increased in length up to 80 cm, remained constant between 80 and 120 cm, and decreased at greater lengths. An extraction coil length of 100 cm (0.5 mm i.d.) was selected.

The volume of sample injected was varied from 35 to 150 μl by changing the length of the sample loop in the injection valve. The peak height increased with increasing sample size up to 50 μl, above which it remained virtually constant. The volume to be injected was selected as 60 μl.

Effect of the reagent concentration

With the concentration of orange IV solution fixed at 5×10^{-4} M, the pH of the buffer solution (carrier) was varied between 2.0 and 5.0. The peak height was maximum and constant from pH 2.3 to 3.1, and decreased outside this range (Fig. 3). Therefore, a 0.5 M chloroacetate buffer of pH 3.0 was used as the carrier. With the pH fixed at 3.0, the concentration of orange IV was varied between 5×10^{-5} and 6×10^{-4} M. The peak height increased with increasing concentration of the dye solution stream up to 2.5×10^{-4} M, but levelled off at higher concentrations. The concentration adopted in the procedure was 3×10^{-4} M.

Analytical features

The effect of the concentration of bromhexine on absorbance was studied by measuring the peak height when 60 μl of bromhexine hydrochloride solution of different concentrations was injected. The calibration graph was found to be linear between 5.0×10^{-6} and 1.6×10^{-4} M, and the regression equation obtained was

$$A = (-0.02 \pm 0.009) + (7400 \pm 103)C \qquad (r = 0.9994)$$

where C is the molar concentration of bromhexine, A is the absorbance and r is the correlation coefficient. The relative standard deviations of ten injections of each solution containing 1.0×10^{-5} and 8.0×10^{-5} M bromhexine were 0.88 and 0.32%, respectively. The lower detection limit, calculated as the value corresponding to three times the standard deviation of the blank, was 5×10^{-7} M of bromhexine. The sampling rate was 40 samples h^{-1}.

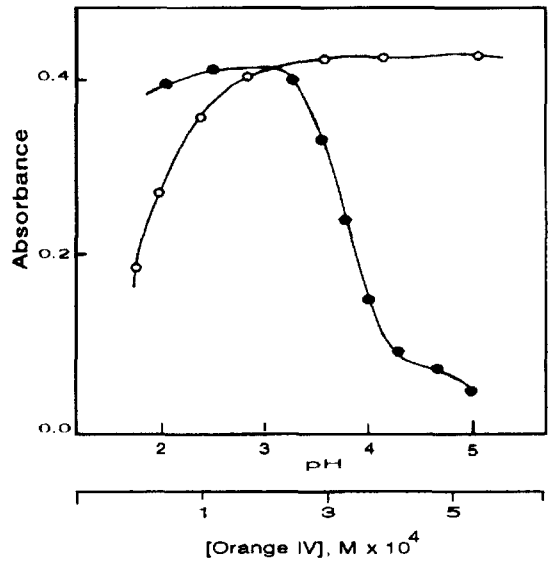

Fig. 3. Effects of pH (●) and orange IV concentration (○) on the peak height.

The selectivity of the methods was studied by preparing solutions containing 8 μg ml^{-1} of the drug and increasing the concentration of the foreign substance up to 800 μg ml^{-1}. The tolerance ratio of each foreign compound was taken as the largest amount yielding an error less than ±4% in the analytical signal of bromhexine. Glucose, sucrose, lactose, saccharin, caffeine, starch, sodium bromide and magnesium nitrate were tolerated in large amounts (100-fold excess was the maximum tested): a 50-fold excess of acetylsalicylic acid and a 10-fold excess of gelatin and citrate were also tolerated.

Applications

The method was applied to the determination of bromhexine in blood serum. The sample of blood serum was collected from a healthy volunteer and bromhexine was added at a similar concentration to those used in clinical applications. The serum was first treated with trichloroacetic acid to separate the proteins; after centrifugation, bromhexine was determined in the centrifugate by the standard-addition method. The recoveries obtained for bromhexine were very good (Table 2).

The method was also applied to the determination of bromhexine in a pharmaceutical preparation (Bisolvom®). The commercial preparation was dissolved in distilled water and bromhexine was analyzed by the general procedure. Table 2 summarizes the results.

Table 2
Determination of bromhexine in real samples

	Added (μg ml^{-1})	Found[a] (μg ml^{-1})	Recovery (%)
Blood serum			
Sample 1	10.0	9.9 ± 0.17	99.0
	20.0	19.9 ± 0.11	99.5
	40.0	40.1 ± 0.26	100.2
	80.0	80.1 ± 0.25	100.1
Sample 2	20.0	19.7 ± 0.10	98.5
	40.0	39.6 ± 0.26	99.0
Sample 3	10.0	10.1 ± 0.20	101.0
	30.0	29.9 ± 0.26	99.6
	50.0	49.7 ± 0.36	99.4
Pharmaceutical preparation			
Bisolvom®[b]		20.1 ± 0.10	
	10.0	30.2 ± 0.36	102.0
	20.0	40.1 ± 0.10	100.5

[a] Mean of three determinations ± SD.
[b] Centrified value: 2 mg ml^{-1}. 100-fold dilution before the analysis.

4. Conclusions

Results of experiments with differnt dyes and solvents for extraction showed that orange IV and 1,2-dichloroethane were the most effective for use in unsegmented flow configurations with a continuous extraction system. This system overcame the complexity of manual extraction methods and avoided problems and hazards involved in handling toxic organic solvents.

It was demonstrated that bromhexine can be determined by a flow-injection method based on the extraction of an ion-pair with orange IV using photometric detection. A comparison of this method (Table 3) with other automatic methods, which use a continuous liquid–liquid extractor coupled on-line to an atomic absorption spectrophotometer for the indirect determination of bromhexine based on the formation of ion-pairs with inorganic complexes [14,15], shows that its sensitivity, dynamic range and throughput are as good, although the detection system is cheaper.

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Table 3
Characteristics of the flow-injection methods for the determination of bromhexine

Parameter	Ion-pairing agent			
	BiI_4^-	$Co(SCN)_4^{2-}$	Reinecke's salt	Orange IV
Sample pH	2.0	1.5	1.5	3.0
Concentration of the ion-pairing agent (mol l^{-1})	2×10^{-3}	0.5	1.5×10^{-3}	3×10^{-4}
Detection system[a]	AAS	AAS	AAS	VS
Linear range (μg ml^{-1})	3–60	15–175	5–120	1.9–60
Slope of calibration graph $\times 10^{-3}$	5.1	0.89	1.75	19.7
Relative standard deviation (%)	2.4	4.6	1.0	0.3
Detection limit (μg ml^{-1})	2.0	5.5	2.8	0.19
Sampling rate (h^{-1})	20/30	20/30	30	40
Reference	[14]	[14]	[15]	This work

[a] AAS = atomic absorption spectrometry; VS = visible spectrometry.

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