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

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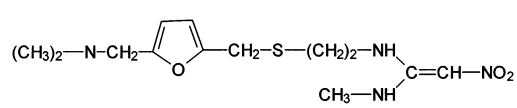
Abstract

The spectrophotometric determination of trace amounts of ranitidine was carried out by liquid–liquid extraction using bromothymol blue with a flow system. The determination of ranitidine in the range of 1×10^{-5}–1×10^{-4} mol l^{-1} was possible with a sampling frequency of 40 samples h^{-1}. The method was satisfactorily applied to the determination of ranitidine in pharmaceutical preparations and the recovery was quantitative and no interferences from excipients were observed. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Ranitidine; Bromothymol blue; Flow-injection; Extraction; Pharmaceuticals

1. Introduction

Ranitidine (*N*-(2-{[5-dimethylamino-methyl]-2-furanil}-methylthioethyl) *N′*-methyl-nitro-1,1′-diaminoethane) was introduced in the market in 1981 and is now extensively used in the treatment of duodenal and gastric ulceration, reflux oesophagitis and dyspepsia [1,2]. It is a histamine H_2-receptor antagonist that, unlike cimetidine that contains an imidazole ring, has a furan ring structure. This substituted aminoalkylfuran derivative is more potent than the cimetidine as an inhibitor of gastric acid secretion, and it is marketed in a range of dosage form including tablets, syrups and injection solutions.



Various analytical techniques have been employed for the quantitative analysis of ranitidine. Potentiometric titration is suitable for the determination of relatively large amounts of the drug [3]. Other methods included polarography [4–7], voltametry [8,9] and potentiometric sensors [10–13]. Several chromatographic techniques such as HPLC [14–23], supercritical fluid chromatography [24] and capillary electrophoresis [25,26] have been used for the determination of ranitidine.

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There are few methods for the spectrophotometric determination of ranitidine. UV spectrometry has been used [27]. The other methods are based on the reaction of ranitidine with some acidic dyes and different reagents such as 3-methylbenzothiazoline-2-ona hydrazone. These methods, however, are not adaptable for use in automated system due to the long reaction time for colour development and involved high reaction temperature [28–31].

There is a constant search for simple, reliable, automated and semiautomated method for the rapid quantification of substances of therapeutic interest in pharmaceutical samples and biological fluids. However, only a few flow-injection methods for the determination of ranitidine have been described in the literature [32–34].

Flow-injection (FI) methodology in association with extraction in organic solvents currently provides a means of automating and speeding up the handling of reagents in routine analysis with good selectivity and sensibility.

The purpose of this work was to investigate the formation and extraction behaviour of ion pairs of ranitidine with acid dyes in order to develop useful automatic spectrophotometric methods. The results showed that bromothymol 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 minimises the above shortcomings as the organic solvents are kept in closed vessels. The proposed automatic method has been applied to the determination of ranitidine in pharmaceutical preparations.

2. Materials and methods

2.1. Reagents

Ranitidine was obtained from Sigma (St Louis, MO, USA) and used as received. A standard 1.0×10^{-3} mol l^{-1} 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} mol l^{-1}) of bromothymol blue, bromophenol blue, orange IV, Methyl orange, bromocresol purple, thymol blue and erythrosin B 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 analytical reagent grade.

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) Miniplus 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. Acetate buffer of pH 5.0 and bromothymol blue solutions were pumped

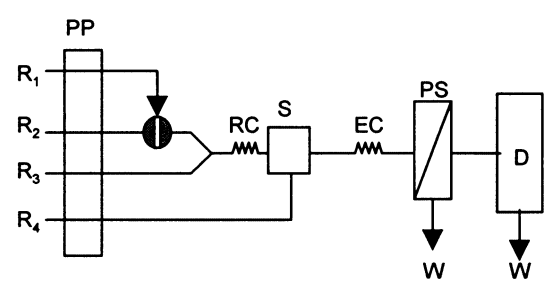

Fig. 1. Manifold for determination of ranitidine: PP, peristaltic pump; R_1, sample; R_2, buffer (pH 5); R_3, bromothymol blue; R_4, chloroform; RC, reaction coil (150 cm $\times$ 0.5 mm i.d.); S, segmentor; PS, phase separator; EC, extraction coil (400 cm $\times$ 0.5 mm i.d.); W, waste; D, detector.

through Tygon tubes and chloroform was pumped through the Acidflex tube. The sample (300 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 extraction coil was 500 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 pins. 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 inside 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 420 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 were finely powdered and weighed. An amount of this powder, equivalent to about 10 mg of ranitidine was accurately weighed and shaken with 20 ml of distilled water and 10 ml of 5 mol l^{-1} 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 mol l^{-1} hydrochloric acid. The combined filtrate and washings was adjusted to pH about 5 with 2 mol l^{-1} sodium hydroxide and diluted with distilled water to 100 ml in a calibrated flask to obtain a solution of 100 μg ml^{-1}.

Table 1
Extraction of ranitidine-dye ion pairs

Dye	A_{blank}	$A_{ion\text{-}pair}$
Bromothymol blue	0.010	0.580
Bromophenol blue	0.000	0.075
Methyl orange	0.004	0.100
Bromocresol purple	0.085	0.430
Orange IV	0.025	0.200
Erythrosin B	0.100	0.110
Thymol blue	0.013	0.012

Ranitidine concentration, 6×10^{-5} mol l^{-1}; dye concentration, 3×10^{-4} mol l^{-1}; A_{blank} values correspond to the absorbances of organic extracts (chloroform) of the samples containing all reagents in the absence of ranitidine.

3. Results and discussion

Ranitidine 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:

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

where RH^{+} and D^{-} represent the protonated ranitidine and the anion of the dye, respectively, and the subscript (aq) and (org) refer to the aqueous and organic phases, respectively.

The dyes studied for ranitidine ion-pair formation were bromothymol blue, bromophenol blue, orange IV, Methyl orange, bromocresol purple, thymol blue and erythrosin B. Of the dyes tested, bromothymol 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. The polarity of the solvent affects both extraction efficiency and absorbance. The results using bromothymol blue are shown in Table 2. In this study, bromothymol 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 ranitidine–bromothymol blue ion-pair

Solvent	$A_{ion\text{-}pair} - A_{blank}$
Chloroform	0.570
1,2-Dichloroethane	0.540
Methyl isobutyl ketone	0.030
Ethyl acetate	0.200

Ranitidine concentration, 6×10^{-5} mol l^{-1}; bromothymol blue concentration, 3×10^{-4} mol l^{-1}.

3.1. Characteristics of the ranitidine–bromothymol blue ion-pair

Bromothymol 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 5 (Fig. 2).

The composition of the ion-pair was established by Job's method of continuous variations [35] and by the molar ratio method [36] using both variable dye concentration and variable ranitidine concentration. The results obtained with these methods showed a molar ratio between dye and ranitidine of 1:1 and an apparent stability constant of $\log K_f = 5.37 \pm 0.30$. The extraction constant for the above equilibrium was $\log K_{ex} = 6.17 \pm 0.30$.

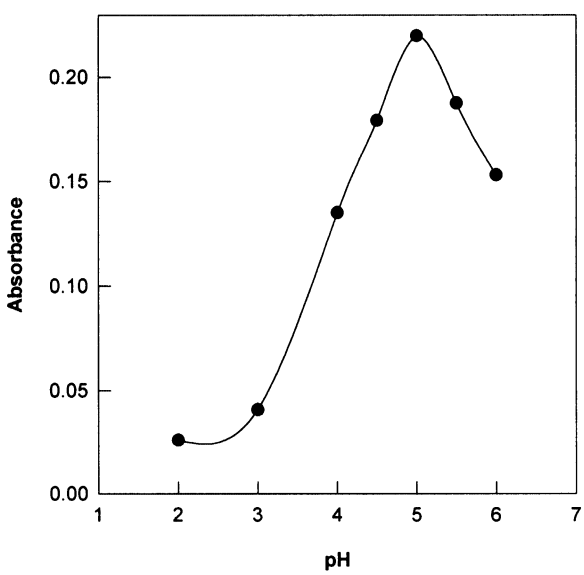

Fig. 2. Influence of pH on the extraction of ion-pair. Ranitidine = 2.4×10^{-5} mol l^{-1}, Bromothymol blue = 1×10^{-4} mol l^{-1}.

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 95.8%.

3.2. Flow-injection determination of ranitidine

The flow manifold (Fig. 1) for the automation of the proposed method was arranged so as to consider the essential features of ranitidine–bromothymol blue ion-pair. The universal buffer Britton–Robinson was replaced by the acetate buffer in order to use a simpler buffer with high buffer capacity at pH 5.

3.2.1. Influence of manifold parameters

The optimisation 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 an acetate buffer of pH 5 (0.2 mol l^{-1}) and the reagent stream was an 1×10^{-4} mol l^{-1} bromothymol blue solution.

The flow-rate of the aqueous and organic streams was varied in order to obtain the maximum concentration coefficient without significantly decreasing the sample throughput. The optimisation of flow-rate resulted in the adoption of 1.1 (0.55 for each channel) and 3.2 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 is formed rapidly.

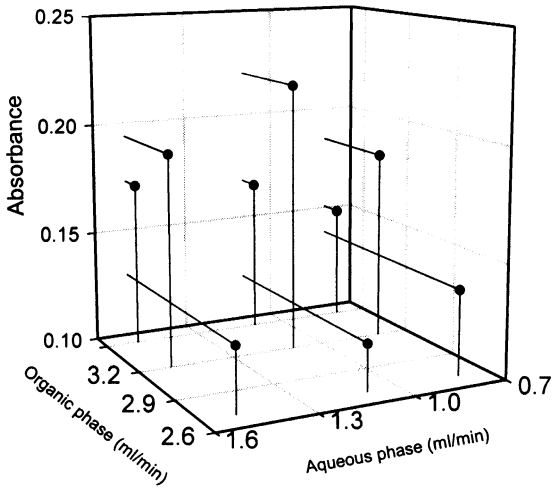

Fig. 3. Influence of flow rate of the aqueous and organic streams on the extraction efficiency of the ranitidine–bromothymol blue ion-pair.

The influence of the extraction coil length was also examined. The peak height increased as the extraction coil increased in length up to 350 cm, above which the signal remained virtually constant. An extraction coil length of 400 cm (0.5 mm i.d.) was selected.

The volume of sample injected was varied from 35 to 350 μl by changing the length of the sample loop in the injection valve. The peak height increased with increasing sample size up to 300 μl, above which it remained virtually constant. The volume to be injected was selected as 300 μl.

3.2.2. Effect of the reagent concentration

Using a 3×10^{-4} mol l^{-1} bromothymol blue solution as reagent stream, the pH of the buffer solution (carrier) increased with increasing pH values from 2 to 5, above which it decreased. Therefore, a 0.1 mol l^{-1} acetate buffer of pH 5.0 was used as carrier. With the carrier stream buffered at pH 5.0, the concentration of bromothymol blue was varied between 3×10^{-5} and 3×10^{-4} mol l^{-1}. The peak height increased with increasing concentrations of the dye solution stream up to 1×10^{-4} mol l^{-1}, but levelled off at higher concentrations. The concentration adopted in the procedure was 1×10^{-4} mol l^{-1}.

3.2.3. Calibration graph and reproducibility

The effect of the concentration of ranitidine on the absorbance was studied by measuring the peak height when 300 μl of ranitidine hydrochloride solution of different concentrations were injected. The calibration graph was found to be linear between 1.0×10^{-5} and 1.0×10^{-4} mol l^{-1} (3.51–35.1 mol l^{-1}), and the regression equation obtained was:

$$A = -(8.0 \pm 1) \times 10^{-4} + (9.3 \pm 0.2) \times 10^{3} C; \quad (r = 0.9982)$$

where C is the concentration of ranitidine in mol l^{-1}, A the absorbance and r the correlation coefficient. The detection limit, calculated to IUPAC recommendations [37], was 5.68×10^{-6} mol l^{-1}. The sampling rate was 40 samples per hour. The relative standard deviation (R.S.D.) of ten injections of each solution containing 7.02 and 24.56 μg ml^{-1} of ranitidine were 0.96 and 0.81%, respectively.

The reproducibility of the method was studied by analysing, on five different days, ten identical solutions of ranitidine (8.0×10^{-5} mol ml^{-1}). Every day three injections of each solution were made; the R.S.D. 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 was studied by preparing solutions containing 5×10^{-5} mol l^{-1} of ranitidine and increasing concentrations of the potential interferent up to 0.01 mol l^{-1}. The tolerance (Table 3) of each foreign compound was taken as the largest amount yielding an error of less than $\pm 3\%$ in the analytical signal of ranitidine.

The robustness of the method was also tested in the presence of foreign substances. Lactose, caffeine and saccharin were selected because their tolerance was high, medium and low, respectively. The within- and inter-day reproducibility was studied following the same conditions

Table 3
Tolerance of different species in the determination of ranitidine

Species added	Maximum tolerable mole ratio
Sucrose, glycerine, sorbitol, sodium fluoride, lactose, fructose, gelatine, mannitol	200[a]
Benzyl alcohol, caffeine, Zn^{2+}	100
Saccharine	20

Ranitidine = 5×10^{-5} mol l^{-1}.
[a] Maximum ratio tested.

as described above. The results obtained are summarised in Table 4.

3.2.5. Analysis of pharmaceutical preparations

In order to establish the validity of proposed method, several pharmaceutical preparations were analysed. The data in Table 4 show that the assay results were in good agreement with the labelled contents

The recovery was determined by adding various amounts of ranitidine to each pharmaceutical preparation and subtracting the results obtained for pharmaceuticals prepared in the same manner but to which no ranitidine had been added. Taking into account that recoveries obtained were close to 100%, it may be assumed that no interfering substances were encountered (Tables 5 and 6).

Table 4
Reproducibility of the method for ranitidine in the presence of foreign substances

Foreign substance	Within-day[a]	Inter-day[b]
Lactose	0.98	1.56
Caffeine	1.24	1.82
Saccharin	1.46	1.98

Ranitidine, 5×10^{-5} mol l^{-1}; lactose, 5×10^{-3} mol l^{-1}; caffeine 2×10^{-3} mol l^{-1}; saccharin, 8×10^{-4} mol l^{-1}.
[a] The values are the R.S.D. of ten injections of each solution.
[b] The values are the R.S.D. of three injections every day on 5 different days.

Table 5
Determination of Ranitidine in pharmaceutical preparations

Preparation	Supplier	Amount found (mg)[a]
Ranuber (150 mg per tablet)	ICN Ibérica	151.3 ± 1.1
Normon (300 mg per tablet)	Normon	299.5 ± 1.9
Zantac (150 mg per tablet)	Glaxo	150.4 ± 1.2
Zantac (50 mg per 5 ml of injectable solution)	Glaxo	49.7 ± 0.8

[a] Mean of seven determinations ± standard deviation.

4. Conclusions

The above results clearly indicate that ranitidine can successfully be determined by extraction as ion-pair with bromothymol 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 the determination of ranitidine in pharmaceutical dosage forms and may be used as a stability indicating procedure.

Table 6
Recovery of Ranitidine

Sample[a]	Added (μg ml^{-1})	Found (μg ml^{-1})[b]	Recovery (%)
Ranuber	5	4.93 ± 0.08	98.6
	15	15.06 ± 0.10	100.4
	20	19.96 ± 0.12	99.8
Normon	5	5.03 ± 0.07	100.6
	15	15.15 ± 0.15	101.0
	20	19.78 ± 0.10	98.9
Zantac (Tablets)	5	4.95 ± 0.06	99.0
	15	15.16 ± 0.11	101.1
	20	19.88 ± 0.10	99.4
Zantac (Ampoules)	5	4.91 ± 0.07	98.2
	15	14.78 ± 0.13	98.5
	20	20.25 ± 0.15	101.2

[a] See Table 4.
[b] Mean of three determinations ± standard deviation.

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