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Optimization and validation of a dissolution test for famotidine tablets using flow injection analysis

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Abstract

A dissolution test for famotidine tablets was optimized and validated using flow injection analysis (FIA). The effect of dissolution parameters such as pH, medium and stirring speed was studied, while the ruggedness of the procedure was validated. All measurements were performed using a simple direct spectrophotometric flow injection assay ($\lambda_{max} = 265$ nm) that has also been optimized and fully validated in terms of linearity, limit of detection, precision, selectivity and accuracy. Linearity was obeyed in the range 50–150% of famotidine (20–60 mg L^{-1}), while the detection limit (0.1 mg L^{-1}) and repeatability ($s_r < 1.0\%$, $n = 12$) were satisfactory. The sampling rate was 30 h^{-1}. The dissolution results during quality and stability control of two batches of famotidine tablets obtained by the flow injection method were in good agreement with high-performance liquid chromatography (HPLC).

Keywords: Dissolution; Flow injection analysis; Famotidine; Validation; Spectrophotometry

1. Introduction

Famotidine belongs to a class of drugs known as histamine H_2 blockers. Famotidine is useful in any situation where stomach irritation is an issue and ulceration is a concern. It is often used in the treatment of helicobacter infection, inflammatory bowel disease, any disease involving protracted vomiting, etc. Compared to its "cousins" cimetidine and ranitidine, famotidine is 32 times stronger in its ability to inhibit stomach acid than is cimetidine and is nine times stronger than ranitidine [1,2].

Although the H_2 blockers group have generally very limited side effects (most commonly headache), there have been some reports of exacerbating heart rhythm problems to patients who already have such problems. Other less common or rare side effects may include from anxiety, nausea, sleepiness to fatigue and muscle and bone pain.

In the pharmaceutical industry, dissolution testing is a very important tool in drug development and quality control. The dissolution characteristics of a solid dosage form (tablets and capsules) of a drug are influential on its in vivo absorption. In other words, the in vitro dissolution behavior of a formulation may be related to the in vivo performance of a drug product [3]. Based on this general consideration, in vitro dissolution tests are used to assess the lot-to-lot quality of a formulation and to guide development of new products [4].

High performance liquid chromatography (HPLC) is usually the technique of choice in pharmaceutical quality control. Based on its separation ability, HPLC offers the potential of both assay and purity control of formulations. However, when it comes to dissolution testing, flow injection analysis is an advantageous analytical technique in terms of rapidity, cost effectiveness and solvent-free wastes. Although several HPLC methods have been reported for the analysis of famotidine formulations [5–17], only two flow injection (FIA) assays were found in literature [18,19]. They are based on the reaction of famotidine with either 2,4-dichloro-6-nitrophenol ($\lambda_{max} = 450$ nm) [18] or cupric acetate ($\lambda_{max} = 630$ nm) [19]. However, these FIA methods are not suitable for dissolution studies of famotidine as either methanolic medium is necessary for reaction development (not compatible to the aqueous dissolution medium) [18], or the determination range is not appropriate (50–500 mg L^{-1}, while the expected theoretical

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concentration of famotidine is ca. 40 mg L^{-1} in the final solution) [19].

The present study has two main objectives. The first is to optimize and validate a simple and rapid FIA assay capable of directly analyzing samples from dissolution experiments of famotidine tablets with accuracy and precision. The method is based on direct, on-line measurement of the absorbance of the analyte at 265 nm. The second objective is to optimize and validate a dissolution protocol for famotidine containing formulations. The effective combination of the dissolution protocol and the FIA assay were used on the production and on-going stability quality control of two batches of famotidine tablets (Ansilan® FC TABS, Cosmopharm Ltd., Korinthos, Greece).

2. Experimental

2.1. Reagents

All chemicals were provided by Merck (Germany), unless stated otherwise. Purified water ($\kappa < 4.3$ μS cm^{-1}) was used for preparing solutions.

Famotidine working standard (Lot No.: F279/QC 2005/14/Assay = 99.20%) was provided by Ercros (Division of Fyse, Madrid, Spain). Standard solutions were prepared by dissolving appropriate amounts of the working standard in either 0.1% CH_3COOH or 0.1 M phosphate buffer (13.6 g of potassium di-hydrogen phosphate per liter adjusted to pH 3 with 1.0 mol L^{-1} HCl).

The pharmaceutical excipients used in the selectivity and accuracy studies (magnesium stearate, maize starch and microcrystalline cellulose) were provided by domestic suppliers. The placebo mixture (all excipients except for the active ingredient) contained per gram: 581.0 mg microcrystalline cellulose, 412.4 mg maize starch and 6.7 mg magnesium stearate.

2.2. Instrumentation

The hardware (pump, injection valve, autosampler and detector) of an HP1100 (Hewlett Packard) instrument was used throughout the experiments. The single-channeled FIA setup is depicted in Fig. 1. A 10-cm long/0.28 mm i.d. PTFE mixing coil was used to connect the injection valve to the detector in flow injection experiments, while a Hypersil BDS C_{18} column (250 mm × 4 mm/5 μm) was used instead for HPLC measurements. Data acquisition (peak height and peak area for FIA and HPLC, respectively) was performed via Chem Station® software.

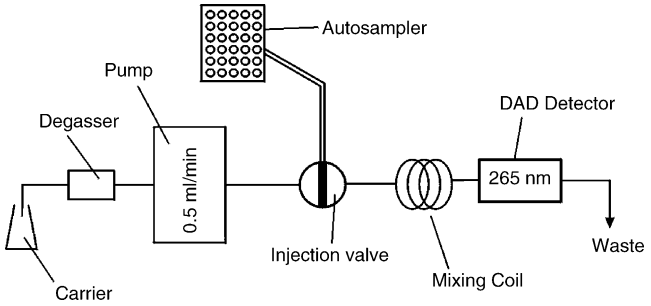

Fig. 1. FIA manifold for the determination of famotidine.

A Distek Premiere 5100 system equipped with an autosampler was used for the dissolution experiments.

pH measurements were performed via a Knick 761 Calimatic (Germany) digital pH-meter, using a Blueline 18 pH (Schott, Germany) electrode. The electrode was calibrated by standard buffers (Reagecon, Spain) prior to use.

2.3. FIA procedure for aqueous solutions

Twenty microliters of famotidine standards (20–60 mg L^{-1}) or dissolution samples were injected via the autosampler to a 0.1 mol L^{-1} phosphate buffer carrier stream. The sample zone was propelled at a flow rate of 0.5 ml min^{-1} through a 10-cm long mixing coil towards the diode-array photometric detector (λ_{max} = 265 nm). Peak height was used for quantitative measurements, while each standard/sample was injected in triplicate. Sharp peaks and stable base-line were observed in all cases.

2.4. Dissolution studies of famotidine tablets

Optimization and validation experiments were performed using Ansilan® tablets (Cosmopharm Ltd., Lot 019, 40 mg famotidine per tab). In each experiment, twelve tablets were weighed and introduced to the dissolution apparatus. The dissolution profile was recorded via automated sampling at 5, 10, 15, 20, 30, 45 and 60 min (n = 7). The temperature was kept constant at 37.0 ± 0.5 °C and the volume of the dissolution medium was 900 mL in all cases. The paddle dissolution approach was used throughout the experiments (at 100 rpm), while the withdrawn aliquots were filtered in-line using 45 μm PTFE disc filters. The dissolution buffer in each vessel was ultrasonically degassed for 15 min prior to use.

2.5. HPLC analysis

The accuracy of the dissolution tests was evaluated by comparison of the FI results to an in-house validated HPLC assay (according to the Drug Master File of the manufacturer of the active ingredient). For this reason, aliquots of the samples collected from the dissolution apparatus were analyzed by HPLC without any further pretreatment. The mobile phase consisted of a (20:80, v/v) mixture of MeOH and buffer solution (2.76 g L^{-1} NaH_2PO_4, pH adjusted to 7.0 with 1.0 mol L^{-1} NaOH). The flow rate was 1.0 ml min^{-1}, the injection volume 25 μL, the column temperature 25 °C and the detection wavelength 265 nm. A Hypersil BDS C_{18} column (250 mm × 4 mm/5 μm) was used throughout the experiments. Under the above-mentioned conditions, the retention time of the analyte was ca. 6 min.

3. Results and discussion

3.1. Optimization of the flow injection assay

The sample-injection volume and the carrier flow rate were studied and optimized at a concentration of 40 mg L^{-1} famotidine. The starting values of these variables were 10 μL and 1.0 ml min^{-1}, respectively.

The effect of the volume of the injected sample was studied in the range of 10–50 μL. A non-linear increase of the signals was observed within that range. This was expected, since the volume of the sample injected in a FIA system is inversely proportional to the dispersion of the sample zone. The value of 20 μL was selected for further experiments as a compromise between sensitivity and linear determination range.

The carrier flow rate (0.2–1.2 mL min^{-1}) had little effect on the sensitivity of the determination, because it does not significantly contribute to dispersion in single-channeled manifolds. However, at 0.5 mL min^{-1}, a better run-to-run reproducibility was obtained. It should be noted that the sampling rate was not affected by the flow rate of the carrier stream. In all cases, the sampling rate was controlled by the cycle injection time of the HP 1100 autosampler, which was 2 min. Thus, the "real" maximum sampling rate was 30 h^{-1}, regardless of the geometry of the FIA manifold (the peaks were completed within 20 s).

3.2. Validation of the flow injection assay

Validation was performed according to the ICH guidelines [20]. The linearity of the FIA method was validated in the range of 20–60 mg L^{-1}. Based on the fact that the strength of the famotidine formulation is 40 mg per tablet and the volume of the dissolution medium is 900 mL, the theoretical expected final concentrations should be around 40 mg L^{-1}. The 20–60 mg L^{-1} range "brackets" the above-mentioned levels, fulfilling the 50–150% linearity criterion. The experimental results showed that the method was linear within the studied range, obeying the regression equation

$$\text{mA.U.} = 10.06(\pm 0.07)\gamma(\text{famotidine}) + 19.54(\pm 2.74)$$

with a correlation coefficient of 0.9999 ($n = 12$). Within-day ($n = 8$) and day-to-day ($n = 8$) experiments (at five famotidine concentrations, namely 20, 30, 40, 50, 60 mg L^{-1}), verified the linearity of the FIA assay. In both cases (within- and day-to-day), the relative standard deviations of the slope and intercept were less than 4.2%.

The detection limit of the developed FIA method was estimated by S/N = 3 criterion. The LOD was calculated to be 0.1 mg L^{-1}, which is satisfactory for this type of analysis.

The within- and day-to-day precision of the proposed assay was studied by performing repeatability experiments at 20, 40 and 60 mg L^{-1} famotidine. The results verified the repeatability of the procedure, since the relative standard deviations were <1.0% for within-day ($n = 12$) and <3.6% for day-to-day ($n = 8$) experiments.

The specificity of the FIA method was evaluated by studying the potential interfering effect of various concentrations of a placebo mixture (a mixture containing all excipients and excluding the active ingredient) at the target famotidine mass concentration of 40 mg L^{-1}. Appropriate amounts of the placebo mixture were dispersed in famotidine standard solutions and sonicated for 15 min. The resulting solutions were analyzed after filtration through 0.45 μm disposable syringe filters. Although the maximum expected placebo concentration in real samples is ca. 230 mg L^{-1}, the selectivity experiments showed no interference even at the maximum tested concentration of 500 mg L^{-1}, which is more than double that of the expected value.

Table 1
Accuracy studies

Synthetic sample	Placebo added (mg L^{-1})	Famotidine added mg per 1000 mL	Recovery (±s), %
1	500	20.2	100.6 (±0.8)
2	500	20.9	101.0 (±0.6)
3	500	41.6	101.4 (±1.1)
4	500	39.8	99.2 (±0.5)
5	500	59.2	100.1 (±0.5)
6	500	60.7	99.7 (±0.9)

Recovery of famotidine from synthetic samples.

In order to validate the accuracy of the assay, placebo solutions (containing 500 mg L^{-1} placebo) were spiked with different amounts of famotidine; 20, 40 and 60 mg L^{-1}. The usual sonication–filtration procedure was followed prior to each synthetic sample analysis. The experimental results are shown in Table 1. The percent recoveries were acceptable in all cases, ranging between 99.2 and 101.4%. Day-to-day accuracy was examined at three different days and from three different analysts. The results are tabulated in Table 2.

3.3. Optimization of dissolution test

The dissolution parameters (pH, medium, stirring speed) were optimized in terms of dissolution rate and precision. The temperature was kept constant at 37.0 ± 0.5 °C and the volume in each vessel at 900 mL. Six tablets were processed in each dissolution experiment.

The effect of the pH was studied at 3.0, 4.5 and 7.0 using 0.1 mol L^{-1} phosphate buffer. The experimental results are

Table 2
Day-to-day accuracy studies

Synthetic sample	Placebo added (mg L^{-1})	Famotidine added mg per 1000 mL	Recovery (±s), %
Day 1/analyst A	500	20.2	100.6 (±0.8)
	500	41.6	101.4 (±0.6)
	500	60.7	99.7 (±1.1)
Day 2/analyst B	500	19.4	98.9 (±1.3)
	500	40.5	99.3 (±0.8)
	500	59.3	101.6 (±0.4)
Day 3/analyst C	500	20.9	100.2 (±1.0)
	500	40.9	101.9 (±2.3)
	500	61.5	100.6 (±1.5)

Recovery of famotidine from synthetic samples.

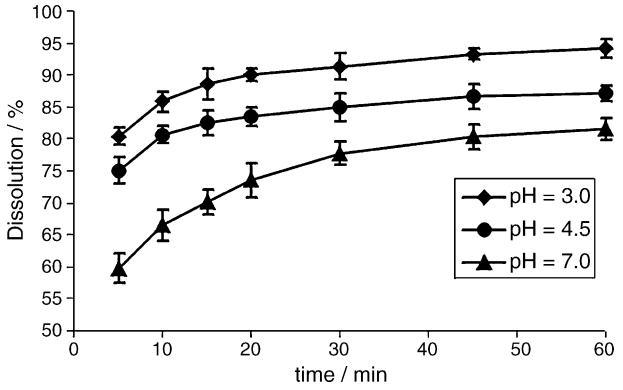

Fig. 2. Effect of the pH of the medium on the dissolution rate of famotidine tablets. $T = 37.0 \pm 0.5\,^{\circ}C$, $V = 900$ mL, paddle rotation speed = 50 rpm.

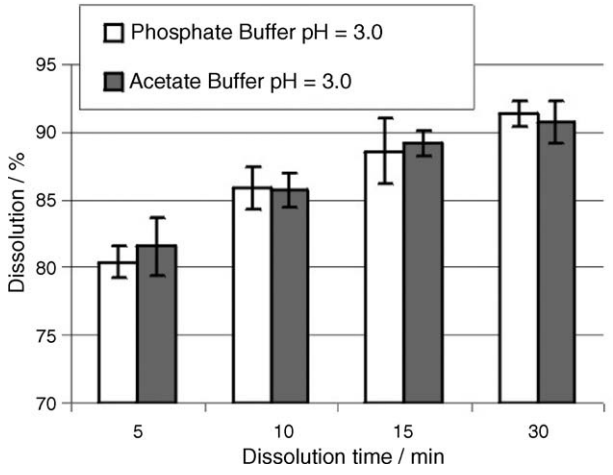

Fig. 3. Effect of the medium species on the dissolution rate of famotidine tablets. $T = 37.0 \pm 0.5\,^{\circ}C$, pH 3.0, $V = 900$ mL, paddle rotation speed = 50 rpm.

summarized in Fig. 2. As can be deducted from Fig. 2, the dissolution rate increases at the more acidic pH, due to higher solubility of the active ingredient. However, the dissolution limits set by the pharmacopoeia (min 75% at 30 min) were fulfilled in all cases. The pH of 3.0 was selected for further studies. It should be noted that at pH 3.0, 100% liberation

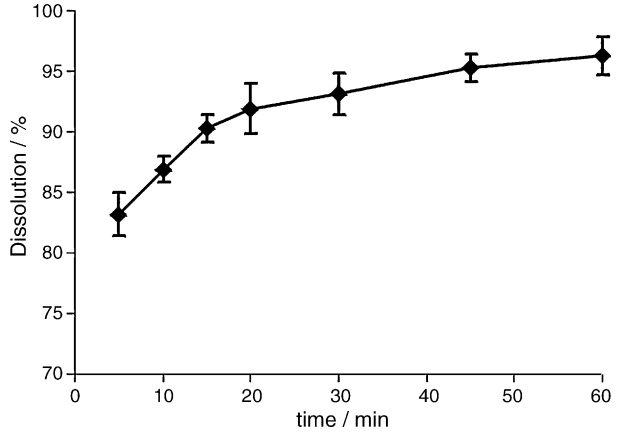

Fig. 4. Dissolution profile of famotidine at pH 1.2. $T = 37.0 \pm 0.5\,^{\circ}C$, $V = 900$ mL, paddle rotation speed = 50 rpm.

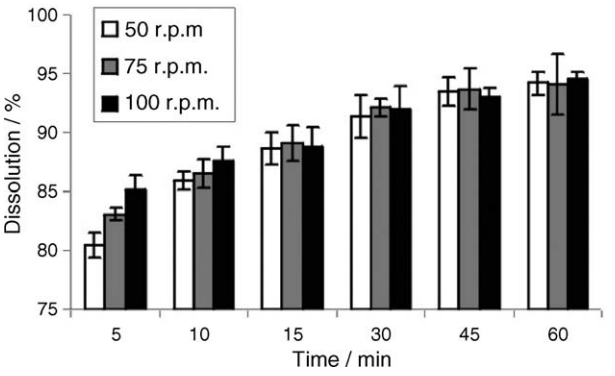

Fig. 5. Effect of the paddle rotation speed on the dissolution rate of famotidine tablets. $T = 37.0 \pm 0.5\,^{\circ}C$, pH 3.0, $V = 900$ mL.

of famotidine was achieved within 120 min of dissolution time.

The effect of the dissolution medium was studied by using an alternative buffer (0.1 mol L^{-1} acetate buffer) at the optimum pH. The results depicted in Fig. 3 indicated no significant effect of the medium species on the dissolution rate of famotidine.

In order to have a complete outlook of the dissolution behavior of famotidine, dissolution experiments were also performed using medium of pH 1.2 (to simulate the pH of the gastric fluids). The pH was adjusted by 6 mol L^{-1} HCl solution. The results were similar to the findings using a dissolution medium of pH

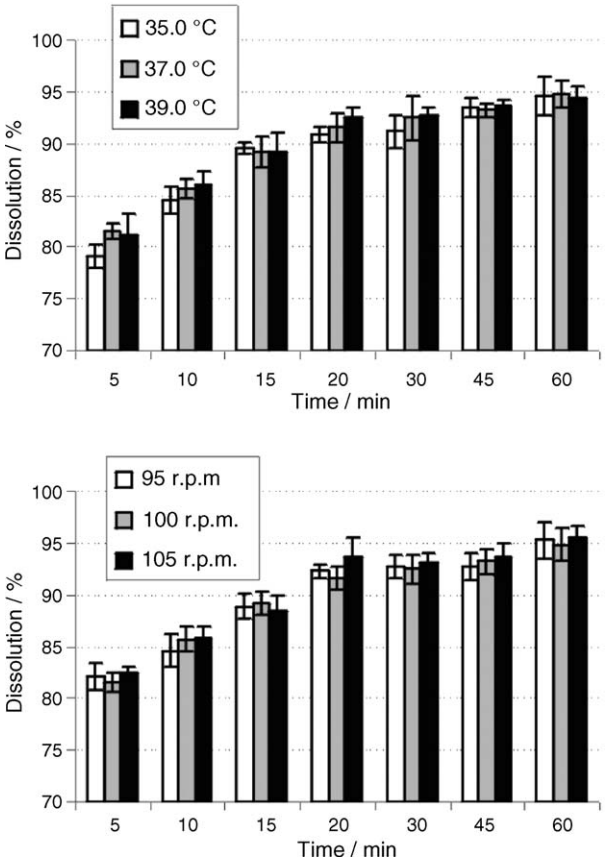

Fig. 6. Ruggedness of the dissolution test against temperature and paddle rotation speed.

Table 3
Dissolution results of Ansilan® tablets

Batch	Percent dissolution (30 min)[a]		
	FIA	HPLC	*t*-value[b]
019 (production)	90.2 ± 1.6	91.3 ± 2.1	0.35
019 (3 months LTS)	91.5 ± 1.4	92.1 ± 1.8	0.26
019 (3 months ACS)	90.0 ± 2.5	90.3 ± 2.6	0.19
019 (6 months LTS)	92.7 ± 1.4	90.5 ± 2.4	0.98
019 (6 months ACS)	89.9 ± 2.3	91.5 ± 2.9	0.76
018 (production)	89.1 ± 1.9	90.7 ± 1.6	0.78
018 (3 months LTS)	90.5 ± 2.1	89.1 ± 2.5	0.81
018 (3 months ACS)	88.2 ± 2.0	90.3 ± 3.1	1.12
018 (6 months LTS)	91.2 ± 1.1	91.7 ± 1.6	0.21
018 (6 months ACS)	90.8 ± 1.8	89.7 ± 2.7	0.73

LTS: long term stability (at $T = 25.0\,^{\circ}C$ and RH = 60%). ACS: accelerated stability (at $T = 40.0\,^{\circ}C$ and RH = 75%).
[a] Mean percent dissolution of 12 tablets per batch or test.
[b] The theoretical *t*-value for 10° of freedom (95% level) is 2.23.

3.0, verifying the solubility of famotidine under acidic conditions (Fig. 4).

The effect of the paddle rotation speed was examined at 50, 75 and 100 rpm. The results are shown in Fig. 5. The effect was noticeable only at 5 and 10 min, were the dissolution rate was favored by the increased rotation speed. Above 15 min the results were practically unaffected by the rotation speed of the paddle. The value of 100 rpm was selected for further experiments.

3.4. Ruggedness of dissolution test

The ruggedness of the dissolution test was examined against small, deliberate variations of some critical parameters such as the pH, temperature and paddle rotation speed.

The pH was varied in the range 2.8–3.2, the temperature in the range 35.0–39.0 °C and the stirring speed in the range 95–105 rpm. The results confirmed the ruggedness of the test, since the variations in the dissolution rate were less than ±5% of that under the optimal experimental conditions. The experimental results are depicted in Fig. 6.

3.5. Application to production and stability quality control of famotidine tablets

The validated flow injection assay and dissolution test were applied to the production and stability quality control of two batches (lots 018 and 019) of Ansilan® tablets (Cosmopharm Ltd., Greece). The samples were also analyzed by a validated HPLC assay. The experimental results are presented in Table 3. In all cases the findings using the FIA assay were statistically equivalent to HPLC based on the *t*-student test and within the limit of 75% (30 min of dissolution time).

4. Conclusions

The present work reports the optimization and validation of a dissolution test for the quality control of famotidine tablets using a new direct flow injection spectrophotometric assay. Both the assay and the dissolution test were optimized and fully validated. Flow injection offered automation, 500% reduction of the analysis time compared to HPLC (2 min versus 10 min) without any loss in accuracy and precision. Additionally, the FIA method is more environmentally friendly, as it produces organic solvents-free waste. All results were within QC-accepted limits ($D > 75\%$ after 30 min) and in good agreement to chromatography.

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