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Short communication

Validation of an HPLC–MS method for rociverine tablet dissolution analysis

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Abstract

The aim of this work was to develop and validate a method to assess the dissolution behaviour of rociverine sugar-coated tablets. In our laboratories, an HPLC–MS in reverse phase method of analysis was developed for the dosage of unknown rociverine solution. This analytical method was applied to determine the dissolution rate of rociverine tablets produced by the industrial procedure, because there is no official method description. Dissolution tests were carried out in sink conditions as follows: dissolution medium HCl 0.01N, paddle rotation speed 50 rpm and vessel volume 1000 ml. The dissolution test gave satisfactory results: 95% of the drug was dissolved within 30 min and drug dissolution was concluded after 60 min. The method was demonstrated to be adequate for Quality Control of rociverine tablets. Validation was inferred from specificity, linearity, precision, accuracy and robustness.

Keywords: Rociverine; Validation; HPLC–MS; Tablet dissolution

1. Introduction

Drug absorption from a solid dosage form after oral administration depends on the release of the drug substance from the drug product, the dissolution or solubilisation of the drug under physiological conditions, and the permeability across the gastrointestinal tract. Because of the critical nature of the first two of these steps, in vitro dissolution may be relevant to the prediction of in vivo performance [1]. Based on this general consideration, in vitro dissolution tests for immediate release solid oral dosage forms are used to assess the lot-to-lot quality of a drug product, guide development of new formulations, ensure continuing product quality and performance after certain changes in the formulation, the manufacturing process, the site of manufacture, and the scale-up of the manufacturing process [1].

Rociverine, 1-hydroxy[1,1′-bicyclohexyl]-2-carboxylic acid 2-(diethyl-amino)-1-methylethyl ester (MW 339.5 Da), is a muscarinic antagonist [2] with antispasmodic properties [3], useful for the treatment of painful-spasm in the urinary, gastroenteric and biliary tract. The molecular formula is given in Fig. 1. The presence of three chiral carbons in the molecule leads to eight stereoisomers. The *cis*-stereoisomers are commonly named rociverine and are used in clinical practise [4]. It is available as tablet in the unit dose of 10 mg.

Rociverine is not included in any Pharmacopoeia and, up to now, the authority did not require results of tablet dissolution for market authorization; thus, there is no official method description. An unpublished gas chromatographic method exists for the determination of tablet strength, but this method cannot be applied for the determination of the amount of rociverine dissolved during the dissolution test because it is not possible to

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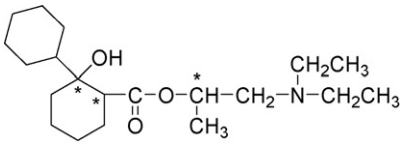

Fig. 1. Chemical structure of rociverine ($C_{20}H_{37}NO_3$).

directly inject the water solution derived from the dissolution test in the gas chromatograph.

The possibility to involve market authorization in new Countries imposes to estimate tablet dissolution profile. The aim of this work is to develop and validate a method to assess the dissolution behaviour of rociverine sugar-coated tablets, adequate for routine Quality Control.

For the evaluation of drug dissolution, a high-performance liquid chromatographic (HPLC) method of analysis was considered. This chromatographic method will be coupled with the mass spectrophotometer (MS) as a detector. Some reports detail the validation of drug dissolution through HPLC methods coupled with either a UV spectrophotometer or a diode array detector (DAD) [5–9]. No examples are reported in the literature concerning validation studies for dissolution tests that couple HPLC with MS or other detectors such as refractive index, electrochemical, or evaporative light scattering.

2. Experimental

2.1. Chemicals

Rociverine ($C_{20}H_{37}NO_3$) (Batch 2006-13800C), which exists as a liquid in ambient conditions, was kindly supplied by Menarini Manufacturing Logistics & Services (Firenze, Italy). All the reagents were of HPLC-grade (Aldrich Chemical, Germany). Ultrapure water was produced by Gradient Milli-Q® (Millipore, Molsheim, France).

The sugar-coated tablets (Rilaten®), containing 10 mg of rociverine, of two different industrial batches, the 06.5 and the 06.7, have been kindly supplied by Menarini and used for the validation of tablet dissolution test. The excipients of the pharmaceutical formulation were citric acid, precipitate silix, bibasic calcium phosphate, cellulose, starch, talc, magnesium stearate, arabic gum, titanium dioxide, saccharose, carnauba wax and blue indigotine.

2.2. Standard preparation for content uniformity

An amount of 14.5 mg of rociverine (averaged MW 339.5; Monoisotopic MW 339.3) was weighed and transferred into a 1000 ml volumetric flask with the minimal amount of acetonitrile, in which rociverine is miscible in all proportions. The solution was then made up to volume with HCl 0.01N (experimental pH 1.81). The final concentration was 14.5 mg l^{-1}. The solution was filtered through a 0.45-μm PTFE membrane filter (Minisart® SRP 4, Sartorius, Hannover, Germany) before analysis.

2.3. Chromatographic instrumentation and conditions

HPLC analysis was performed by a HPLC 1090 Hewlett Packard Series I (Ramsey, MN, USA), equipped with a Hewlett Packard HP1100 MSD Chemstation Rev. A.08.03. The column was a Zorbax SB-Aq, 5 μm, 4.6 mm × 150 mm (Agilent, Santa Clara, CA, USA). The mobile phase consisted of Acetic acid:CH_3CN 40:60 (v/v). The experimental pH of the mobile phase was 3.72. pH was checked by a pH meter (Jenway, Essex, England), equipped with a pH electrode (Jenway, Essex, England) calibrated with appropriate standard solutions. The column temperature was 25 °C. The volume of injection was 10 μl and the flow rate 1 ml/min. The mass spectrometer, performed with an electrospray ionization source (ESI), and analysis were carried out in positive-ion monitoring, SIM, ion:340.2, Frag 75 V, ion source 13.0 l/min drying gas flow, nebulizer pressure 60 psi, drying gas temperature 350 °C, capillary voltage 4000 V.

2.4. Dissolution test conditions

Tablet dissolution test was performed in a multibath ($n = 6$) dissolution test system Erweka (Gloucestershire, England) (DT6), in accordance with the United States Pharmacopoiea (USP) general methods [10]. The apparatus 2 (paddle apparatus) was used with a stirring speed of 50 rpm, selected according to the recommended range (50–75 rpm) for this type [1]. Prior to analysis, rociverine sink conditions were determined in 1000 ml of chloridic acid 0.01N, using an amount of drug equivalent to three times the dose used in the pharmaceutical formulation.

Each rociverine tablet was dissolved in 1000 ml of HCl 0.01N as dissolution medium thermostated at 37.0 ± 0.5 °C. Two millilitres were withdrawn at regular intervals and replaced by fresh medium. The solutions were filtered through 0.45 μm PTFE membrane filter (Minisart® SPP 4, Sartorius, Hannover, Germany) before analysis.

The concentration of unknown rociverine solutions was calculated based on the calibration curve previously determined (peak area = 276681.44 × concentration (mg l^{-1}) + 899734.99; $R = 0.998$). The percentage released was determined based on rociverine tablet strength. The tablet strength of both rociverine batches was previously evaluated by Menarini during the normal Quality Control analysis. The determination was carried out on 20 units and was based on a gas chromatographic method developed and validated in the Menarini's Laboratories. The method is not published. The tablet strength of rociverine batches was 10.0 and 9.7 mg/tablet, respectively for the batch 06.5 and 06.7.

3. Results and discussion

Several chromatographic conditions and detectors were tested during the preliminary phases of this study. Lack of chromophore groups in the rociverine molecule limits the use of more common detectors, such as the diode array detector for the quantitation of unknown rociverine solutions during dissolution. In addition, the RID (refractive index detector) did not reveal sufficiently sensitive for the same objective. In the present

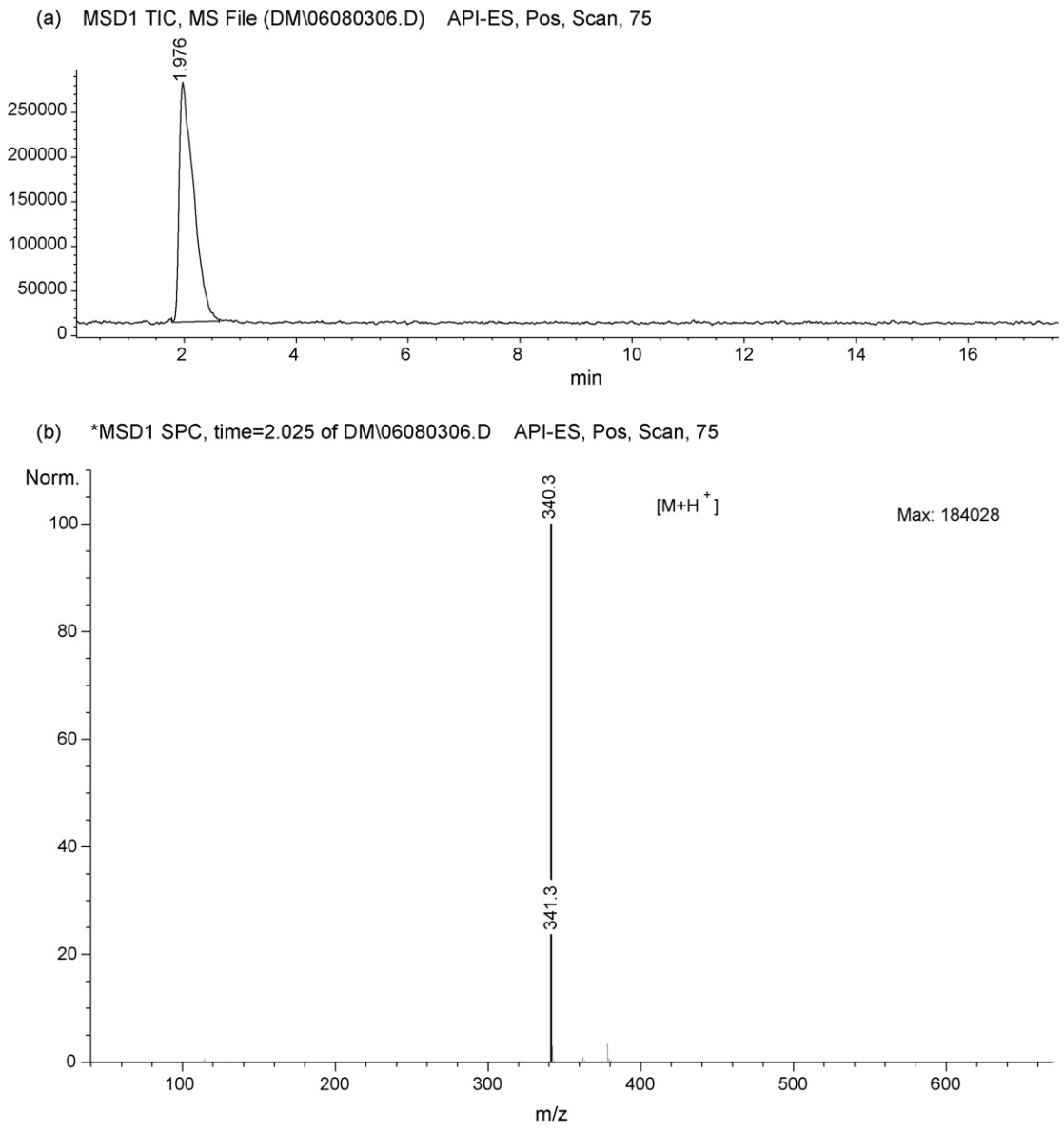

Fig. 2. HPLC chromatogram (a) and full scan-mass spectrum (b) of rociverine in acetonitrile solution.

section, results of a developed and validated method using an HPLC–MS system are described.

Because rociverine is not freely miscible with water, it was initially added in an acetonitrile solution and analysed by HPLC–MS. In Fig. 2(a), the HPLC chromatogram of rociverine in acetonitrile solution is given: one peak can be detected, the retention time of which is 1.98 min. The full scan-mass spectrum of the peak identified by HPLC in acetonitrile is pertinent to the molecular weight of rociverine (Fig. 2(b)): one ion is present corresponding to the protonic adduct of rociverine. Because in vivo rociverine tablets dissolve in stomach and thus in an acidic medium, tablet dissolution was evaluated in acidic conditions [1]. When rociverine is dissolved in acidic conditions (chloridic acid 0.01N; experimental pH 1.8), two peaks appear, with retention times of 1.77 and 2.02 min (Fig. 3(a)). Both peaks identified by HPLC can be attributed to rociverine by mass spectra, because they are pertinent to the molecular weight of rociverine (Fig. 3(b)). It is necessary to be more precise about the fact that during these preliminary studies, the mass analysis was carried out "*in scan*" conditions, while the subsequent dissolution analyses were carried out "*in SIM*" conditions in order to increase the analysis sensitivity and specificity.

In order to illustrate the appearance of the two different peaks in the chromatogram under acidic conditions, several rociverine solutions were prepared by varying pH between 0.5 and 4.0. Particular care was taken in order to minimize the variability in the prepared solutions. For this, a stock solution of 7.18 mg l^{-1} of rociverine in acetonitrile was prepared. Two hundred and eighty microlitres were withdrawn by a Gilson pipette and transferred in a volumetric flask where they were diluted with a certain amount of water:acetonitrile 50:50 (v/v) solution. HCl 1.0N was then added drop by drop in order to reach a specific pH. Water:acetonitrile 50:50 (v/v) solution was added up to the volume of 10 ml. Eight solutions of different pH were prepared: 0.5,

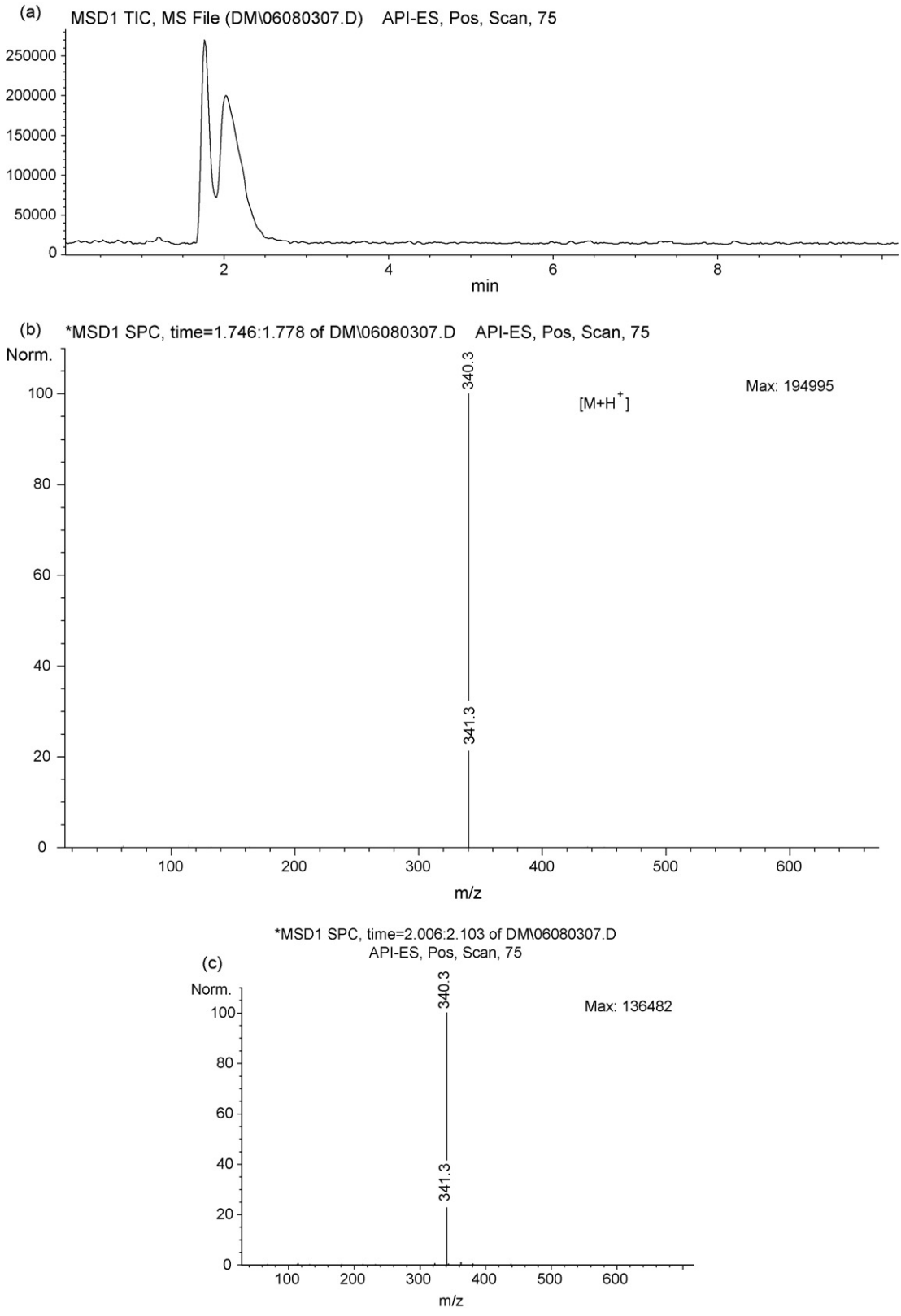

Fig. 3. HPLC chromatogram (a) and full scan-mass spectra of rociverine in HCl 0.01N (b, c), (b) full Scan-mass spectrum relative to the peak at 1.77 and (c) full Scan-mass spectrum relative to the peak at 2.02.

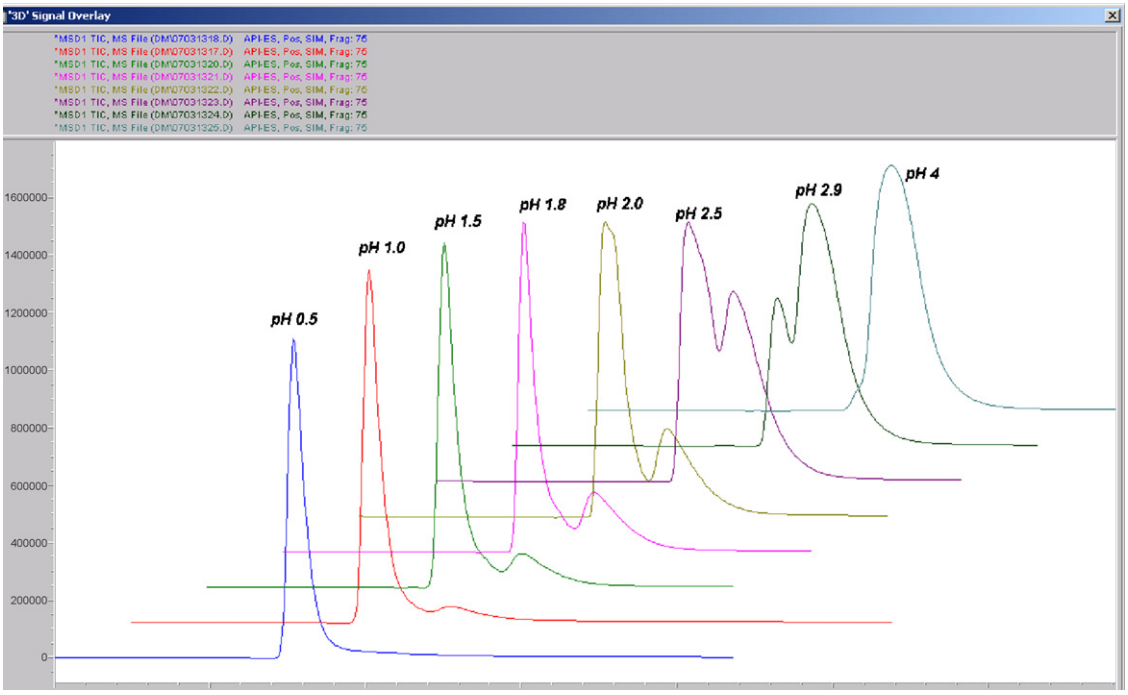

Fig. 4. Superimposed chromatograms of rociverine solutions of different pH.

1.0, 1.5, 1.8, 2.0, 2.5, 3.0 and 4.0. In Fig. 4, the chromatograms of these different solutions are given.

A single peak appears for pH values of 0.5 at the retention time of 1.77 min. By increasing pH, the peak at 1.77 min is accompanied by the appearance of a small shoulder at 2.02 min. The intensity of this peak progressively increases with pH increase. At pH 4.0, only the peak at 2.02 min can be observed. It is of relevant importance that the total area of the peaks for any chromatogram is pertinent to rociverine concentration. For the reasons discussed above, authors selected pH 2 as a dissolution medium. For the quantitative analysis and validation, the two peaks will be always considered as a sum and not separately calculated in order to reduce errors due to integration peaks.

The dissolution method of rociverine was thus validated through the analysis of specificity, linearity, precision, and accuracy parameters.

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present [11]. It was evaluated by studying the potential interfering effect of a placebo sample (a mixture without drug containing all excipients in their usual concentration). The specificity analysis revealed that formulation excipients did not interfere with the HPLC method, because no additional peaks were present in the HPLC chromatogram as a consequence of the injection of a placebo solution eluted for 30 min (Fig. 5).

In order to evaluate *linearity*, aliquots of a 14.5 mg l^{-1} solution of rociverine reference standard prepared in HCl 0.01N were transferred into volumetric flasks and made up to volume with HCl 0.01N in order to obtain final concentrations of 1.45, 2.90, 4.45, 7.25, 10.40 and 12.50 mg l^{-1}. Final concentrations were selected in order to respect the 15–125% linearity criterion. The detection limit was calculated from the injected amount of rociverine which resulted in a peak three times higher than the baseline noise (S/N = 3). The detection limit corresponds to a concentration of 0.07 mg l^{-1}.

Each solution was prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The method demonstrated

Table 1
Summary of the output of the ANOVA for the evaluation of linearity

ANOVA

	d.f.	SS	MS	*F*	Significance *F*
Regression	1	7.22941E+12	7.23E+12	2768.176	7.81122E−07
Residual	4	10446454650	2.61E+09		
Total	5	7.23985E+12			

	Coefficients	S.E.	*t*-Stat	*P*-value	Lower 95%	Upper 95%	Lower 95%	Upper 95%
Intercept	899734.9869	40008.4876	22.4886	2.32E−05	788653.3874	1010817	788653.387	1010816.59
X-variable 1	276681.4398	5258.7580	52.6135	7.81E−07	262080.7567	291282.1	262080.757	291282.123

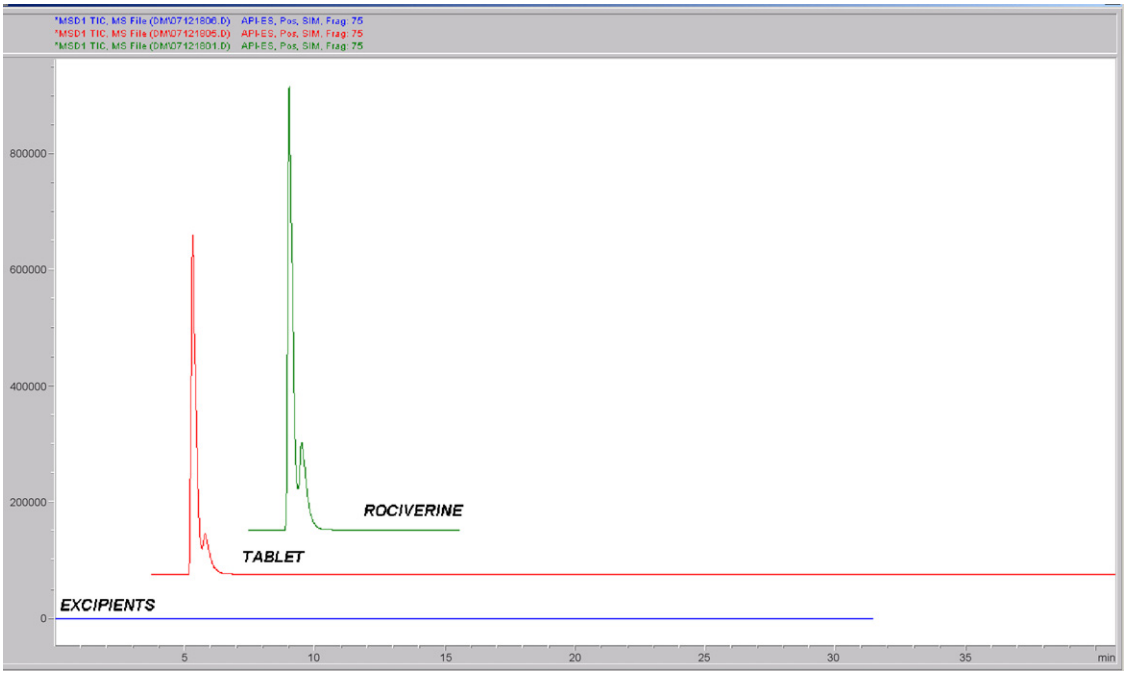

Fig. 5. Method specificity for rociverine demonstrated through the chromatograms of pure rociverine, excipients without rociverine and rociverine tablet.

Table 2
Intra- and inter-day precision for two intermediate rociverine standard solutions

Rociverine concentration ($mg\,l^{-1}$)	Intra-day ($n=5$)		Inter-day (2 days, $n=5$ each)	
	Measured concentration (mean ± S.D., $mg\,l^{-1}$)	R.S.D. (%)	Measured concentration (mean ± S.D., $mg\,l^{-1}$)	R.S.D. (%)
4.45	4.45 ± 0.45	9.9	4.46 ± 0.43	9.6
10.40	10.40 ± 0.38	3.7	10.36 ± 0.38	3.7

to be linear, with a correlation coefficient of 0.998. The slope obtained was 276681.44 and the intercept was 899734.99.

The data were validated by means of analysis of variance (ANOVA), which showed significant linear regression and no-significant linearity deviation ($P<0.05$) (Table 1).

The *precision* was evaluated through the intra- and inter-day values by repeated injections ($n=5$) on two different concentration levels (4.45 and 10.40 $mg\,l^{-1}$), and through the evaluation of S.D. and R.S.D. Results are given in Table 2.

The *accuracy* was inferred from precision, linearity and specificity data, according to ICH Q2B 1996 [12].

The robustness of the method was evaluated through deliberate changes in the mobile phase composition, acetic acid:CH_3CN 42:58 and 38:62 (v/v). Retention times and peak asymmetries are given in Table 3. During these modifications the retention times of the two rociverine peaks undergo very small variations and the symmetry of the peaks is conserved, indicating the robustness of the method.

The drug release profile of the two industrial batches, determined by the dissolution test at mentioned conditions is given in Fig. 6. The results are expressed as rociverine concentration dissolved versus time. The profiles can be considered satisfactory. The dissolution is quite fast: 95% of the drug was dissolved within 30 min and the drug dissolution was concluded after 60 min. FDA specifications [1] state that for highly soluble and rapidly dissolving drug products (which is the case of rociverine) a single-point specification is suitable as a routine Quality Control test, and generally the 85% dissolution value is considered. Thus, it was extrapolated from both curves and the 85% of dissolution is reached within 15 min for both batches. In addition, it must be considered that dissolution curves of the two batches are very similar and the small standard deviations indicate the method *repeatability*. Standard deviations (S.D.) were very low, always comprised between 0.06 and 0.88.

Table 3
Results of the robustness evaluation of the chromatographic method for rociverine

	Mobile phase acetic acid:CH_3CN (v/v)		
	40:60	38:62	42:58
Retention time (min)			
1st peak	1.77	1.73	1.85
2nd peak	2.02	2.05	2.00
Asymmetry			
1st peak	1.78	1.56	1.78
2nd peak	1.75	1.71	1.59

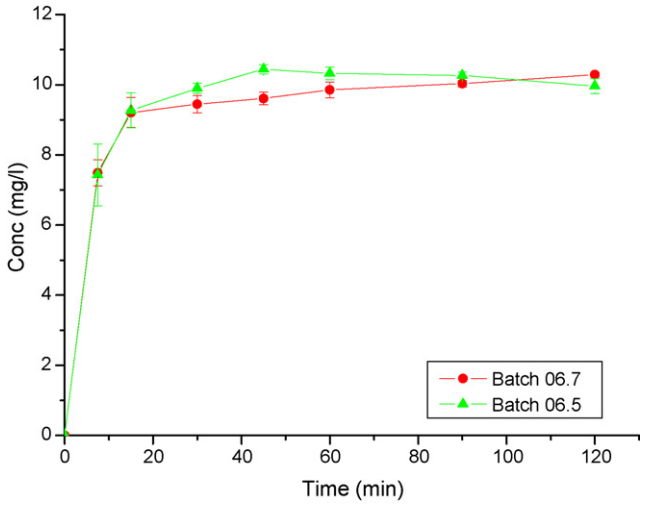

Fig. 6. Dissolution of tablets of two different industrial batches of rociverine.

4. Conclusions

The present work reports the optimization and the validation of a dissolution test for sugar-coated rociverine tablets, based on a new HPLC–MS method of analysis for unknown rociverine solutions. Both the HPLC–MS analysis and dissolution test were fully validated through specificity, linearity, precision, accuracy and robustness, resulting adequate for routine Quality Control of rociverine tablet dissolution. During preliminary studies, attempts were made to use other detectors than MS, but only this one coupled to the HPLC result able to detect and quantify the rociverine, because of the absence of chromofore groups in the molecule.

These results indicate that the LC–ESI-MS single quadrupole can be a very interesting technique for routine Quality Control in pharmaceutical industry.

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