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Ranitidine hydrochloride: development of an isocratic stability-indicating high-performance liquid chromatographic separation

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Abstract

The development of a stability-indicating assay for ranitidine hydrochloride using a mobile phase added ion-interaction reagent was achieved. The assay easily separated all known and unknown impurities/degradants. This assay may be used for the determination of purity, identity and strength for the active ingredient and finished dosage forms. Placebo samples were analyzed for all of the dosage forms and did not interfere with the separation. The system was found to be linear over a range of 0.056 to 44.4 μg/g, with a limit of detection of 0.028 μg/g and a limit of quantitation of 0.056 μg/g. The system precision was determined to be 0.7%. The development of the stability-indicating assay and the effect that each chromatographic variable had on the separation will be discussed.

Keywords: Stability studies; Method development; Ranitidine hydrochloride

1. Introduction

One of the most important aspects of an analytical laboratory in the pharmaceutical industry is the development of a rugged, stability-indicating assay for the identity, purity and strength of an active ingredient in the bulk drug and finished product that can be successfully transferred to another laboratory. In order to ensure that this has been accomplished, steps must be taken during the method development process to demonstrate the reproducibility and ease of use for the assay. Impurities and degradants need to be characterized and separated from the active ingredient as well as from other potential impurities/degradants.

In this study, a high-performance liquid chromatographic separation was developed for the chromatographic purity test of ranitidine hydrochloride {*N*-[2-([{5-[(dimethylamino)methyl]2-furanyl}methyl]thio)ethyl]-*N′*-methyl-2-nitro-1,1-ethenediamine hydrochloride}, a H_2-receptor antagonist used in the treatment of duodenal and gastric ulceration [1]. The current USP method [2] for related compounds employs the use of a thin-layer chromatographic assay for the active ingredient in both the bulk drug and the finished dosage forms. Several analytical techniques have been used to assay ranitidine including a spectrophotometric assay [3], colorimetric assay [4,5], potentiometric techniques [6], and chromatography using an ion-pairing agent [7,8] and reversed-phase high-performance liquid chromatography (HPLC) [9–12]. None of these techniques, however, may be considered stability-indicating and used for impurity/degradation profiles. Spectrophotometric and potentiometric methodologies, in most cases, are not able to distinguish an impurity from the active ingredient. The chromatographic methods used for ranitidine were

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not able to separate all of the impurities/degradants that may be present in the active ingredient.

The goal of this study was the development of a stability-indicating HPLC assay that could be used for the determination of impurities and degradants present in both bulk drug and finished products. A HPLC assay was developed that separated all potential impurities and degradants where an ion-interaction reagent, sodium dodecyl sulfate (SDS), was added to the mobile phase. The mobile phase parameters that may influence the separation were studied and include: the concentration of ion-interaction reagent, the concentration of organic modifier, ionic strength, pH, the base used to adjust the buffer pH, and the column temperature. Additionally, the active ingredient, finished dosage forms and placebos were placed under stressed conditions (acid, base, oxidizing agent, UV light and heat) that would provide evidence that the separation is stability-indicating. The results obtained from these studies are discussed.

2. Experimental

2.1. Reagents and instrumentation

Ranitidine hydrochloride, USP impurities A, B, and C were obtained from Uquifa (Barcelona, Spain). Phosphoric acid, sodium hydroxide, triethylamine, methanol and acetonitrile were purchased from Fisher Scientific (Fairlawn, NJ, USA). HPLC-grade water was obtained by passing deionized water through a Nanopure II water purification system (Barnstead, Dubuque, IA, USA). The instrumentation consisted of a Thermo Separations SCM1000 degasser, a P4000 quaternary pump, an AS3000 variable-loop autosampler with a built-in column oven, a UV6000 photodiode array detector, and a ChromQuest Data System (Thermo Separation Products, San Jose, CA, USA). The YMC-Pack ODS-AM column (150 mm×4.6 mm I.D., 5 μm) and guard column (20×4.0 mm, 5 μm) were purchased from YMC (Wilmington, NC, USA).

2.2. Procedures

Several standards were prepared at a concentration of 1 mg/g in Nanopure-grade water–methanol (94:6, v/v). The working standards were prepared by diluting the 1 mg/g standards with Nanopure-grade water–methanol (94:6, v/v). A sample size of about 100 μg/g was typically used for all studies. A flow-rate of 1.0 ml/min was used for all separations with UV detection at 228 nm. A column temperature of 35°C was used with an injection volume of 25 μl. All mobile phases were prepared by adjusting the pH before the organic modifier was added.

3. Results and discussion

The retention of charged organic ions on reversed stationary phases from a mobile phase containing a hydrophobic ion of opposite charge is determined by two major equilibria [13–20]. The first accounts for the retention of the hydrophobic ion on the stationary phase, while the second describes the ion-exchange selectivity between the analyte ion and the counterion associated with the retained hydrophobic ion. These equilibria, for an alkyl sulfate (RSO_4^-) salt as the mobile phase additive, are shown by Eqs. (1) and (2), respectively:

$$A + RSO_4^-C^+ \rightleftharpoons A\cdots RSO_4^-C^+ \quad (1)$$

$$A\cdots RSO_4^-C^+ + X^+ + Cl^- \rightleftharpoons A\cdots RSO_4^-X^+ + C^+ + Cl^- \quad (2)$$

In these equations, A is the stationary phase, C^+ is the counterion provided by the RSO_4^- salt, the buffer, and/or ionic strength salt, and X^+ is the analyte ion.

The development of a stability-indicating HPLC separation requires that known impurities/degradants be separated from the active ingredient. Fig. 1 shows the structures of rantidine and three potential impurities/degradants. Impurities A and B are highly retained on reversed-phase packings when compared to the retention of either ranitidine or impurity C. Mobile phase conditions that would elute impurities A and B in a reasonable amount of time yet would show retention for impurity C and ranitidine would require the use of an ion-interaction reagent (IIR) to be added to the mobile phase. The IIR produced an increase in retention of impurity C and ranitidine so

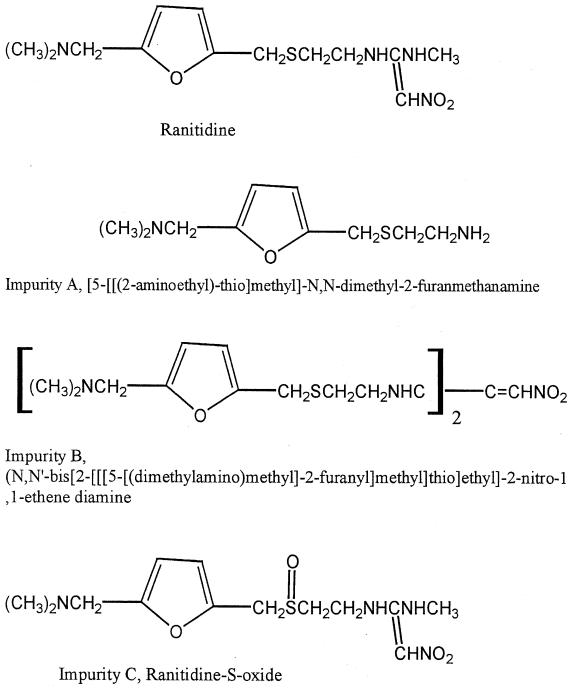

Fig. 1. The structures of ranitidine and potential impurities/degradants.

that an isocratic separation could be accomplished yet impurities A and B would still have a reasonable retention time (under 60 min).

The stability-indicating separation was found to be influenced by the mobile phase ionic strength, mobile phase pH, concentration of SDS, the base used to modify the pH, and the column temperature. Each parameter was studied and the results were used to develop an optimized stability-indicating separation. The effect that each parameter had on the separation of ranitidine from potential impurities/degradants is discussed in detail.

3.1. Effect of sodium dodecyl sulfate concentration

The effect that the mobile phase concentration of SDS had on the ranitidine separation was studied. It had already been determined that without SDS, impurity C had little or no retention on an ODS packing using mobile phase conditions that allowed impurities A and B to elute in under an hour. Therefore, SDS was added to the mobile phase in order for impurity C to be retained.

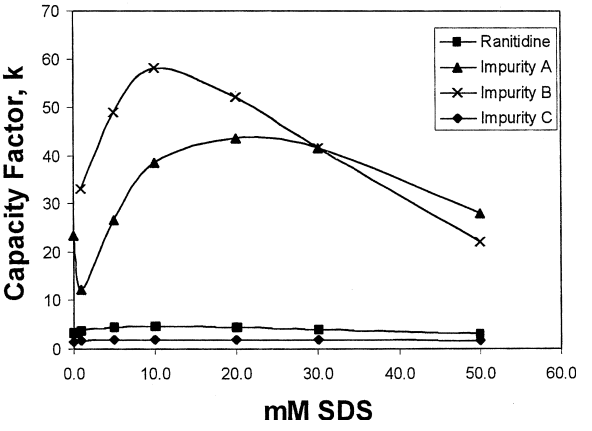

Fig. 2. The effect of the mobile phase concentration of sodium dodecyl sulfate on analyte retention. (Mobile phase: SDS, 50 m*M* H_3PO_4, pH 6.8, 43% methanol).

Fig. 2 shows the effect that the concentration of SDS had on analyte retention. The mobile phase consisted of SDS, 50 m*M* H_3PO_4, pH 6.8 adjusted with triethylamine (TEA), and 43% methanol. Each of the analytes reached a maximum retention over the SDS range studied. Ranitidine, impurity C and impurity B had a maximum retention at 10 m*M* SDS while impurity A reached a maximum retention at 20 m*M* SDS. When the SDS concentration was raised to 30 m*M*, impurities A and B co-eluted. Above 30 m*M* SDS, impurity A eluted after impurity B. This indicates that the SDS influenced the retention of impurities A and B as well as the less-retained impurity C and ranitidine. A mobile phase concentration of 10 m*M* SDS was chosen based on the retention and resolution between these analytes. It was also found during this study that the peak shape of impurity A improved considerably when SDS was added to the mobile phase. Before the addition of SDS, impurity A showed significant peak tailing. The peak tailing may be attributed to hydrogen bonding between the free silanols on the stationary phase and the amine functional groups of impurity A. The improved peak shape may be due to the following: (1) when SDS was added to the mobile phase, the SDS provided a competing site for interactions with the free amine groups; (2) the adsorbed SDS may have helped to block the free silanols and prevented hydrogen bonding with the free amine groups of impurity A.

3.2. Effect of organic modifier

The amount of organic modifier present in the mobile phase will influence the adsorption of the SDS onto the stationary phase and this will in turn affect the retention of the charged analytes that interact ionically with the SDS. Additionally, analytes that are retained predominantly by adsorption will be influenced by the amount of organic modifier. Fig. 3 shows the results that were obtained over a methanol range of 35 to 55% present in the mobile phase (10 m*M* SDS, 50 m*M* H_3PO_4, pH 6.8 adjusted with TEA). The retention of impurities A and B decreased by nearly an order of magnitude over this range, while the retention of impurity C decreased by a factor of two and ranitidine decreased by approximately a factor of three.

This data was used to determine an optimal amount of organic modifier that should be used for the separation. It was determined that 43% methanol would provide a good compromise between the retention of impurity C and the void volume yet would allow impurities A and B to elute in under 60 min.

3.3. Effect of mobile phase pH

The effect that mobile phase pH had on analyte retention was studied and the results are shown in Fig. 4A. The mobile phase consisted of: 10 m*M* SDS, 50 m*M* H_3PO_4, pH adjusted using TEA, 43% methanol. The pK_a of ranitidine is about 2.2, which means that ranitidine will not be ionized over the pH range used in this study. As the mobile phase pH was increased, all of the analytes decreased in retention until pH 8 was reached where retention leveled off.

In order to raise the pH of the buffer, higher concentrations of TEA were required. At first it was believed that the higher TEA concentrations required to raise the buffer pH may be producing an effect

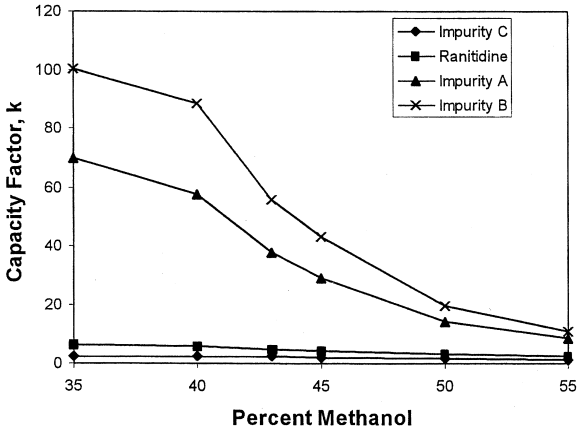

Fig. 3. The effect of the organic modifier concentration on analyte retention. (Mobile phase: 10 m*M* SDS, 50 m*M* H_3PO_4, pH 6.8, methanol).

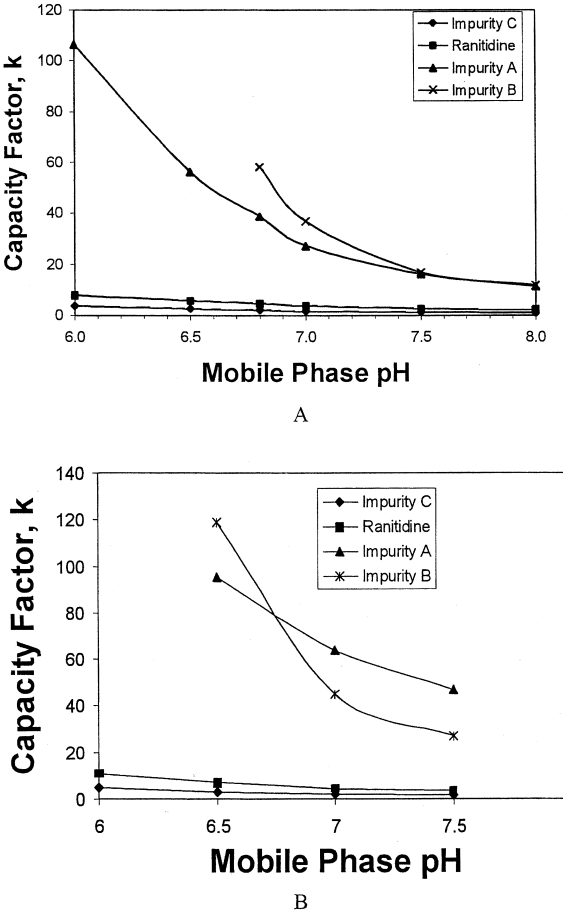

Fig. 4. The effect of mobile phase pH on analyte retention. (A) Triethylamine was used to adjust the buffer pH (mobile phase: 10 m*M* SDS, 50 m*M* H_3PO_4, pH, 43% methanol). (B) Sodium hydroxide was used to adjust the buffer pH (mobile phase: 10 m*M* SDS, 50 m*M* H_3PO_4, pH, 43% methanol).

similar to what an organic modifier would. Therefore, a second study was done to determine if the decrease in analyte retention versus pH was truly a pH effect or that the TEA was acting like an organic modifier. The second study used the same mobile phase conditions that the TEA adjusted mobile phases did except sodium hydroxide was used to adjust the buffer pH. Fig. 4B shows how the sodium hydroxide adjusted pH mobile phases influenced analyte retention. When a comparison was done on retention time, ranitidine and the impurities had much higher retention when sodium hydroxide was used to adjust the buffer pH. For example, at a mobile phase pH of 6.5, impurity A had a *k* of 56.2 with the TEA adjusted mobile phase while the sodium hydroxide mobile phase produced a *k* of 95.4. All of the other analytes showed similar results. Another difference was that at about pH 6.8 for the sodium hydroxide adjusted mobile phase, an elution order change took place between impurities A and B. From this data it appears that the mobile phase concentration of TEA affected the separation in three distinct ways: (1) by providing stronger competition than NaOH for the fixed ion-exchange sites, (2) the higher concentrations of TEA required to raise the mobile phase pH means that more TEA is available to compete for the ion-exchange sites, (3) TEA is acting like an organic modifier with the highly retained analytes which produced a decrease in retention.

Another observation was made when comparing peak shape for the two different bases used to adjust buffer pH. The mobile phases that contained TEA had much better peak shape than the sodium hydroxide mobile phases. This improved peak shape may be due to the TEA blocking the fixed silanol sites better then sodium hydroxide did. From this data it was determined that a mobile phase containing TEA provides better peak shape, analyte retention and resolution than NaOH did. Therefore TEA is the base of choice for adjusting the buffer pH. Additionally, a mobile phase pH of 6.8 was also chosen since it provides good retention for impurity C as well as an acceptable runtime of less than 60 min for the separation.

3.4. The effect of phosphoric acid concentration

The effect that the buffer concentration would have on analyte retention was determined. In this study, the concentration of phosphoric acid over a range of 5 to 100 m*M* was evaluated (Fig. 5). The mobile phase consisted of: 10 m*M* SDS, phosphoric acid, pH 6.8 adjusted with TEA, and 43% methanol. Not only was the concentration of phosphoric acid increased, so was the associated countercation, TEA. Again, a twofold effect took place during this study. First, there was increased competition for the ion-exchange sites associated with the SDS which results in a decrease in the retention of analytes that are retained via ion exchange. Secondly, the increasing TEA concentration required to adjust the buffer pH is also acting like an organic modifier on the analytes that are retained by adsorption, or retained by a mixed mode of adsorption and ion exchange. These two influences produced by the increasing TEA concentration would explain why the analytes showed a decrease in retention over the entire buffer concentration range especially for the more highly retained impurity A. From this data, it was determined that a 50 m*M* phosphoric acid concentration would provide an acceptable separation.

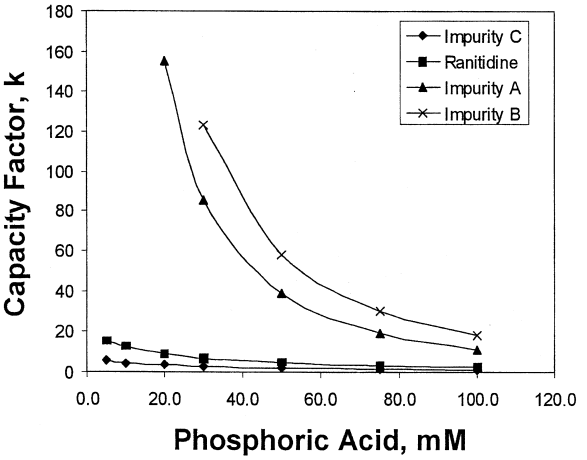

Fig. 5. The effect of the buffer concentration on analyte retention. (Mobile phase: 10 m*M* SDS, H_3PO_4, pH 6.8, 43% methanol).

3.5. Effect of column temperature

Temperature control is an important aspect for ensuring that separations will be consistent from

laboratory-to-laboratory. Separations that use ion-interaction reagents should always have the columns thermostatted [21,22]. If a column is not properly thermostatted, retention time variations may occur.

Ionic samples are easily influenced by changes in temperature due to the different retention processes that may be involved in the separation [23–26]. These influences may include: changing ionization of analytes, hydrophobic retention of charged versus uncharged form, silanol interactions, and changes in buffer pH. Additionally, an increase in the column temperature of 1°C will decrease retention by 1 to 2% [23–26].

A column temperature study over the range of 30 to 55°C (mobile phase: 10 m*M* SDS, 50 m*M* H_3PO_4, pH 6.8 using TEA, 43% methanol) was done to determine how the separation would be influenced. The data that was obtained is shown in Fig. 6. These results were found to be similar to that discussed by Snyder et al. [23–26]. As the column temperature was increased, a corresponding decrease in analyte retention was observed. Temperature control was shown to be important to this separation, therefore a column temperature of 35°C was chosen. This temperature is high enough to ensure: (1) that fluctuations in room temperature will not affect the separation, (2) good resolution between impurity C and the void volume disturbance, and (3) provide a runtime under 60 min.

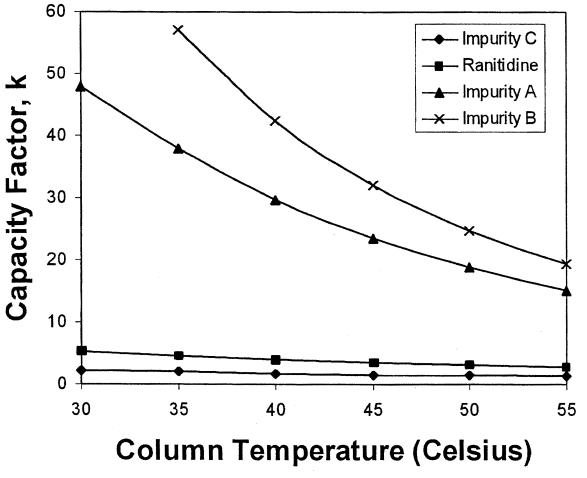

Fig. 6. The effect of column temperature on analyte retention. (Mobile phase: 10 m*M* SDS, 50 m*M* H_3PO_4, pH 6.8, 43% methanol).

3.6. Separations

The optimized separation for ranitidine and potential impurities/degradants is shown in Fig. 7A. The mobile phase consisted of 10 m*M* SDS, 50 m*M* H_3PO_4, pH 6.8 adjusted using TEA and 43% methanol with a column temperature of 35°C. The separation was good for ranitidine and impurities A, B and C with good resolution between impurity C and the void volume disturbance as well as runtime under 60 min. A standard of ranitidine at a concentration of about 10 μg/g was injected into the HPLC system and the result is shown in Fig. 7B.

A typical ranitidine active ingredient sample was analyzed using this separation and is shown in Fig. 8A. Very low levels of impurities A, B and C were observed along with several minor impurities. All of these analytes were at levels below 0.05% (w/w). Fig. 8B shows the analysis of an off-white sample that was degraded for several weeks under fluorescent lighting. A very low level of impurity B was observed, with larger amounts of impurities A and C. A large peak at about 27 min was observed and has been identified as the degradant associated with off-white active ingredient. An increase in the amount of this degradant during a stability study provides a good indication that the active ingredient may be turning off-white.

Additional studies were also done using acid, base, peroxide, heat and UV light. The stress conditions are used to determine if the method can separate all potential degradants for the active ingredient and if so, the separation is considered stability indicating. A 1.0 *M* HCl solution and a 1.0 *M* NaOH solution were allowed to degrade the active ingredient for 1 day while a 1.0% (v/v) hydrogen peroxide solution was allowed to interact with the active ingredient for 4 h. Active ingredient was placed in a 105°C oven for 7 days while a sample of active ingredient was placed into a 500-W UV light chamber for 30 days before analysis. At each stress condition ranitidine was separated from all degradants and the UV spectra of ranitidine showed that the active peak was pure. This data indicates that the separation is stability-indicating.

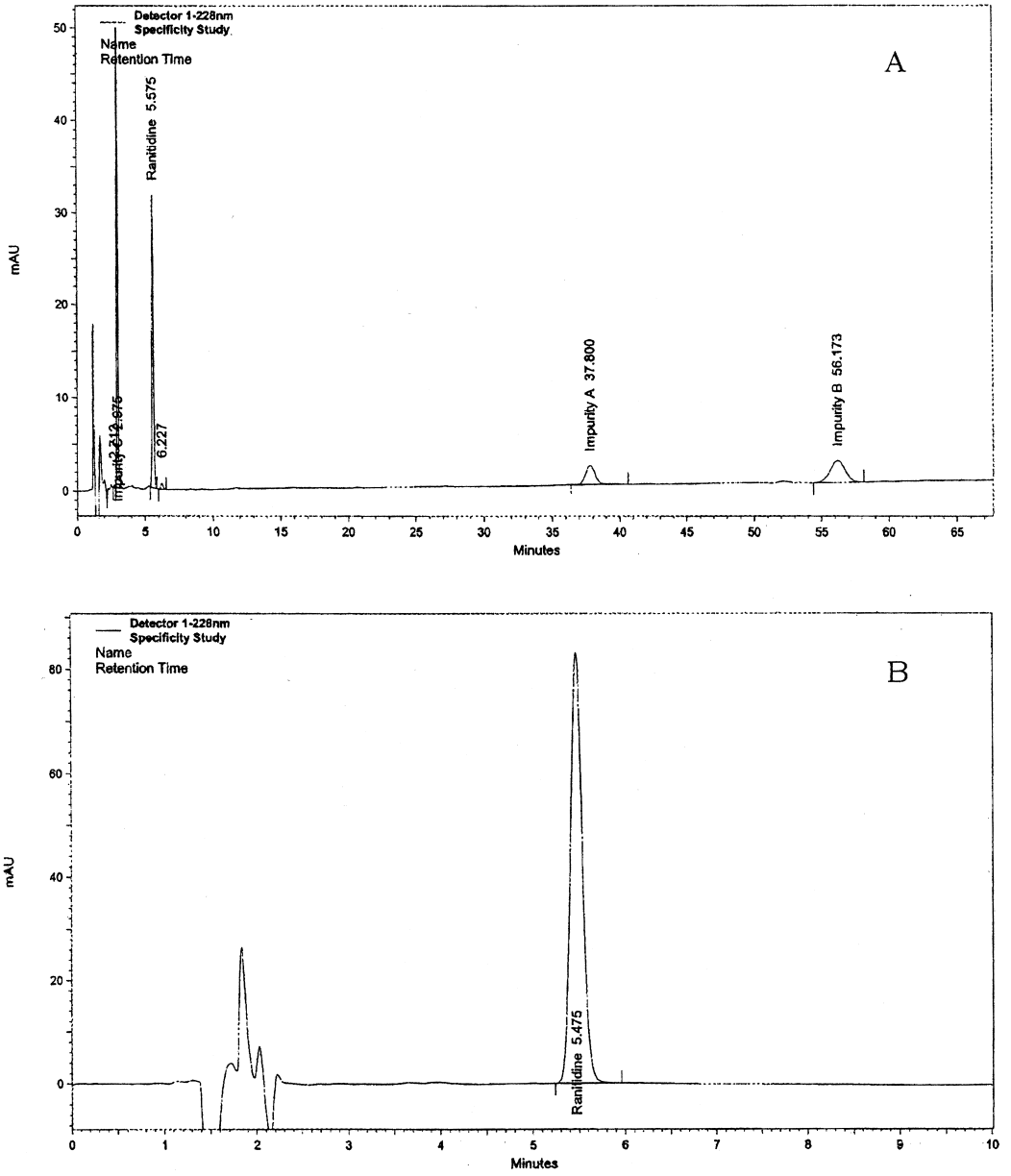

Fig. 7. (A) The optimized separation for ranitidine and impurities A, B, C. (Mobile phase: 10 m*M* SDS, 50 m*M* H_3PO_4, pH 6.8, 43% methanol). (B) The separation of a 10 μg/g ranitidine standard. (Mobile phase: 10 m*M* SDS, 50 m*M* H_3PO_4, pH 6.8, 43% methanol).

3.7. Calibration curves

Calibration curves were established for ranitidine over the range of 0.056 to 44.4 μg/g. A correlation coefficient of greater than 0.9996 was found. The limit of detection was determined to be 0.028 μg/g (3:1 signal:noise) and the limit of quantitation was 0.056 μg/g (10:1 signal:noise). System precision

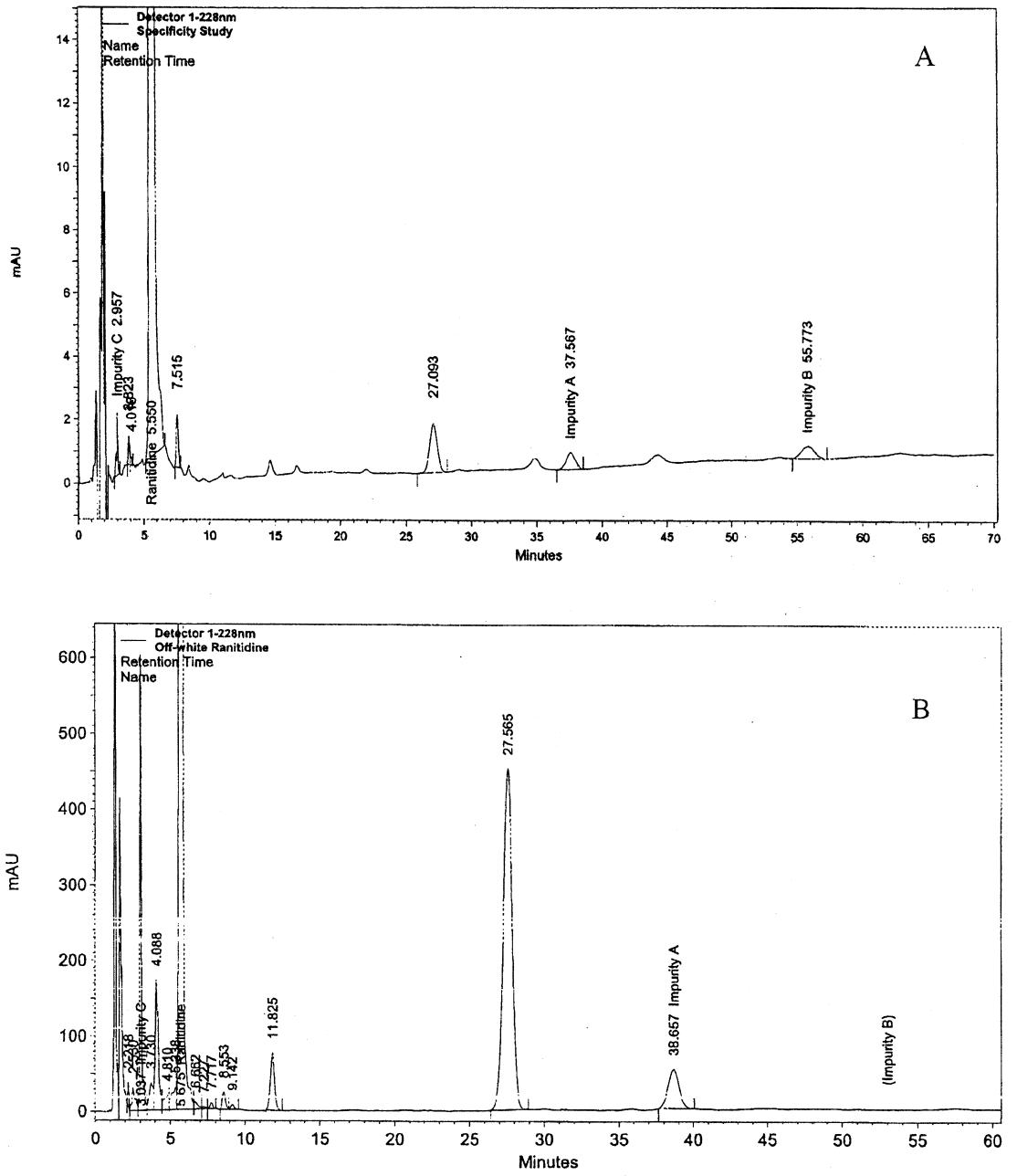

Fig. 8. (A) The analysis of a ranitidine active ingredient sample. (Mobile phase: 10 m*M* SDS, 50 m*M* H_3PO_4, pH 6.8, 43% methanol). (B) The analysis of an off-white ranitidine active ingredient. (Mobile phase: 10 m*M* SDS, 50 m*M* H_3PO_4, pH 6.8, 43% methanol).

was found to be 0.7%. Method precision was under 2.0%. These validation studies were performed according to the Novartis Guidelines.

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