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ORIGINAL ARTICLE

Sensitive liquid chromatography–tandem mass spectrometry method for the determination of pantoprazole sodium in human urine

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Abstract A sensitive and selective liquid chromatographic–tandem mass spectrometric (LC–MS–MS) method was developed to determine pantoprazole sodium (PNT) in human urine. After solid-phase extraction with SPE cartridge, the urine sample was analysed on a C_{18} column (symmetry 3.5 μm; 75 mm × 4.6 mm i.d) interfaced with a triple quadrupole tandem mass spectrometer. Positive electrospray ionization was employed as the ionization source. The mobile phase consisted of acetonitrile–water (90:10, v/v). The method was linear over a concentration range of 1–100 ng mL^{-1}. The lower limit of quantitation was 1 ng mL^{-1}. The intra-day and inter-day relative standard deviation across three validation runs over the entire concentration range was $< 10.5\%$. The accuracy determined at three concentrations (8.0, 50.0 and 85.0 ng mL^{-1} PNT) was within $\pm 1.25\%$ in terms of relative errors.

1. Introduction

Pantoprazole sodium sesqui hydrate (PNT) is chemically known as sodium 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-p-methyl]sulfinyl]-1H-benzimidazole sesqui hydrate (The Merck Index, 1997). Pantoprazole inhibits H^+/K^+ AT Pase pump function thereby reducing the gastric acid secretion and healing the acid related conditions. It is used in the treatment of gerd and peptic ulcer. PNT like omeprazole and lansoprazole also has a role in the eradication of *Helicobacter pylori* infection (Current Index of Medical Specialities, 2005).

The literature survey reveals that only one method available for characterization of pantoprazole impurities by HPLC–MS (Reddy et al., 2007), and few methods are available for the

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determination of PNT in dosage forms by HPLC with UV detection.

Ding et al. (2006) have reported chiral HPLC method for the determination of PNT. In their method PNT was determined on Chiral column (5 μm, 150 mm × 4.6 mm) with methanol:water (35:65, v/v) as mobile phase. The flow rate was 0.6 mL/min. Enantiomeric separation of PNT has also been reported by Ding et al. (2004). They have used hexane–isopropanol–acetic acid mixture (95:5:0.1, v/v) as the mobile phase at a flow rate of 2.0 mL/min at 25 °C. Xue-Hui et al. (2000) have reported determination of PNT in capsules by HPLC on a C_{18} column with the mobile phase of MeCN–phosphate buffer (35:65), and UV detection at 288 nm and voltammetry technique (Nevin, 2003) and HPLC method (Patel et al., 2007). The reported linearity is in the range of 20–60 μg mL^{-1}. All the above cited methods describe the determination of PNT in either dosage forms or pure forms. Now a days liquid chromatography–tandem mass spectrophotometry (LC–MS–MS), due to its higher sensitivity and selectivity, has been applied to the quantification of drug in biological samples. The literature survey revealed that no method has been reported for the determination of PNT in body fluids.

The literature survey reveals that only few methods are available for the determination of PNT in dosage forms in HPLC.

The present study was undertaken to develop a sensitive and rapid LC–MS–MS method for the determination of PNT in urine sample using lansoprazole as an internal standard (Fig. 1). The sample preparation procedure was simple and a run time of each sample was 4.0 min.

2. Materials and methods

2.1. Apparatus

The LC–MS–MS analysis was performed with an API 2000 (Applied Biosystems) coupled to an HPLC system comprising an Agilent 1100 series low pressure quaternary gradient pump with degasser, autosampler, and the column oven.

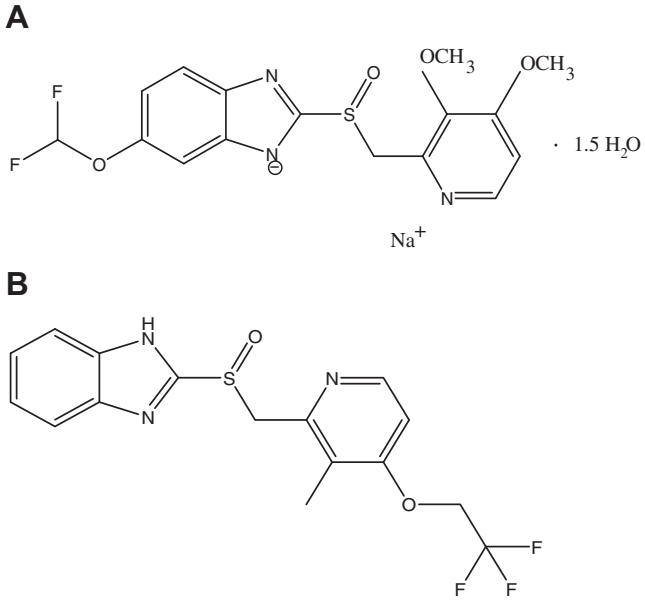

Figure 1 Structure of pantoprazole sodium (A) and internal standard (B).

2.2. Reagents and standards

All chemicals used were of analytical reagent grade and HPLC grade acetonitrile and methanol (Merck. Ltd., Mumbai) was used. Distilled water filtered through 0.22 μm filter (Millipore) was used to prepare the solutions.

Milli Q water and acetonitrile was used as mobile phase A and mobile phase B, respectively. Methanol was used as the diluent.

Pharmaceutical grade PNT, certified to be 99.8% pure was procured from Cipla India Ltd., Mumbai, India, and was used as received. A stock standard containing 100 μg mL^{-1} PNT solution was prepared by dissolving accurately weighed 10 mg of pure drug in diluent and diluting to 100 mL in a calibrated flask with diluent. It was subsequently diluted to obtain a working concentration of PNT.

2.3. Procedures

2.3.1. LC–MS conditions

The chromatographic separation was achieved at ambient temperature (25 °C) on the column (Symmetry C18, 3.5 μm; 75 mm × 4.6 mm i.d) using the mobile phase of water and acetonitrile in the ratio of 10:90 at a flow rate of 0.6 mL min^{-1}. The mobile phase was degassed before use.

Quantitation was performed using multiple reaction monitoring (MRM) of the transitions of m/z 382 → m/z 230 for pantoprazole and m/z 370.4 → m/z 252 for lansoprazole as internal standard (IS) with a scan time of 0.2 s per transition. Fig. 2 shows the product ion spectra of $[M+H]^+$ for pantoprazole and lansoprazole.

In order to optimize all the MS parameters, a standard solution (1 μg mL^{-1}) of the analyte and IS was infused into the mass spectrometer.

Analysis was performed in positive mode (ESI) with a turbo ion spray interface under the conditions: ion source potential, 5500 V, declustering potential, 70 V, focusing potential, 400 V, capillary temperature, 350 °C; entrance potential, 10 V with nitrogen as nebuliser gas at 25 Psi. The column eluent was introduced into the electron spray ionization chamber of the mass spectrometer with a split ratio of 3:7. Mass fragmentation studies were performed by maintaining the normalized collision energy at 23 eV.

2.4. Sample preparation

The urine samples collected from healthy volunteers were spiked with known concentration of PNT and IS and the resulting sample was diluted 1:1 with water prior to loading on SPE cartridge (Oasis HLB). The SPE cartridge was conditioned with methanol and water, and then diluted urine was loaded on. The cartridge was then washed with 5% methanol and then eluted with 100% methanol. Sample was evaporated with nitrogen at 40 °C. and reconstituted the residue with water:methanol (1:1) to 500 μL. Then 20 μL of the above solution was injected into LC–MS–MS for analysis. This procedure was carried out under yellow monochromatic light.

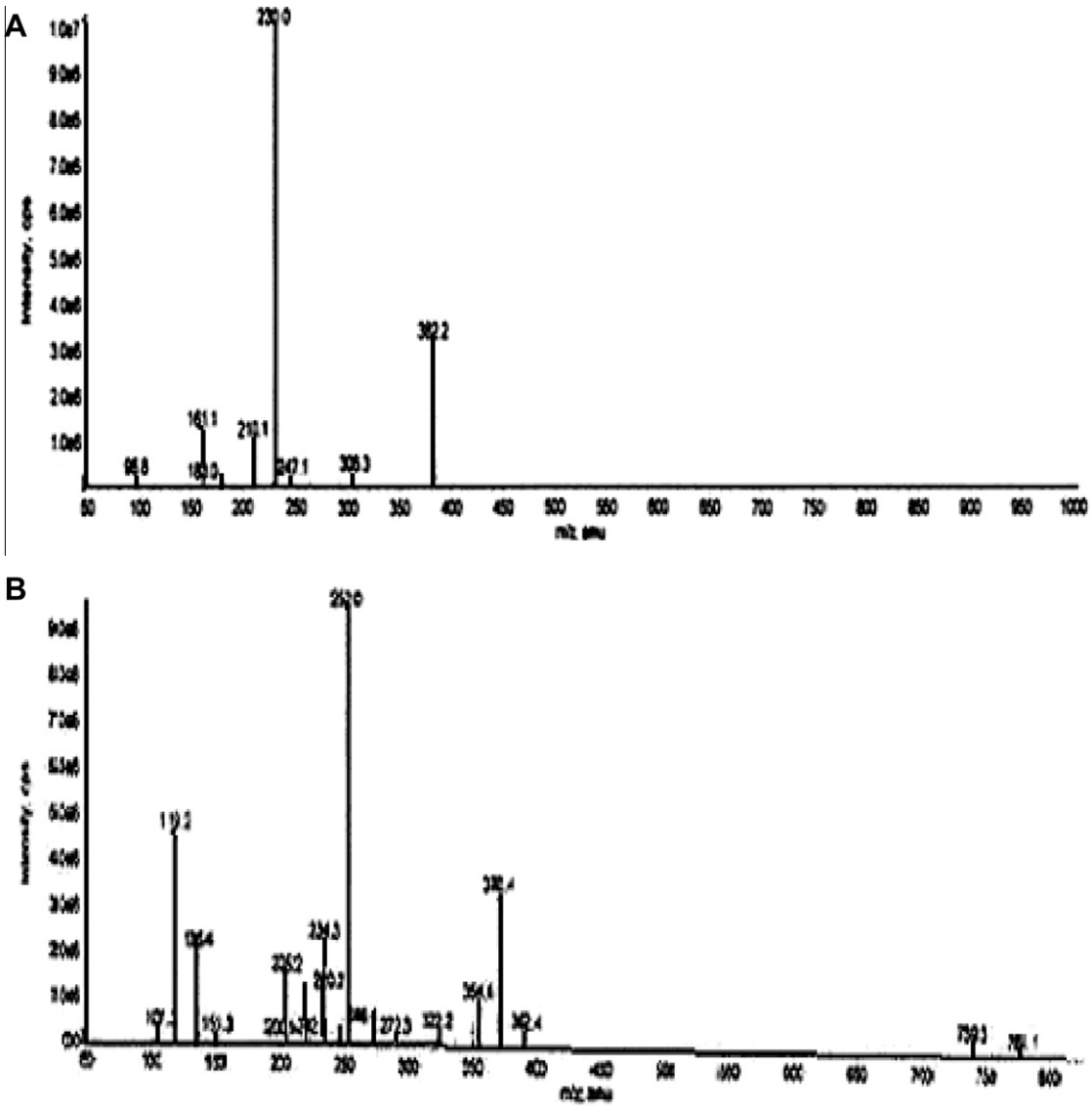

Figure 2 Full scan product ion spectra of pantoprazole (A) and lansoprazole (B).

2.5. Preparation of standard and quality control samples

Stock solution of PNT was prepared in methanol at the concentration of 10 μg mL^{-1}. Stock solution of IS was prepared in methanol at the concentration of 10 μg mL^{-1} and diluted to 500 ng mL^{-1} with methanol:water (1:1). The stock solutions were prepared in amber colored bottle and were stored in refrigerator.

Calibration curve was prepared by diluting the stock solution with methanol:water (1:1) to get appropriate concentration. From these dilutions, 20 μL of appropriate standard solution was added to 1 mL of urine blank to get an effective concentration of 1, 2, 10, 50, 80 and 100 ng mL^{-1} for PNT.

The quality control (QC) samples were separately prepared in the blank urine sample at the concentrations of 8, 50 and 85 ng mL^{-1}, respectively. The spiked urine samples (standards and quality controls) were then treated for SPE.

3. Results and discussion

3.1. Mass spectrometry

The signal intensity obtained for PNT in positive mode was much higher than that in negative mode. Then, the possibility of using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) sources under positive ion detection mode was evaluated during the early stage of method development. ESI spectra revealed higher signals for the molecule compared to APCI source. Further assay development was therefore limited to ESI source. The Q1 full spectra of PNT and IS were dominated by protonated molecules $[M+H]^+$ and no significant solvent adduct ions and fragments ions were observed. The tuning of the ESI source such as capillary temperature, flow of sheath and auxiliary gas (N_2) and spray voltage on the transition of PNT and IS further improved the sensitivity.

3.2. Chromatography

Although in the aspect of chromatographic separation the determination of the analyte was not interfered with by endogenous substances in the urine sample, yet the ionization of the analyte, especially of low concentration, was easily suppressed, which resulted in the linearity of narrow concentration range. In order to avoid the ion suppression induced by endogenous substances, the influence of the mobile phase that composed of different percentage of organic phase to the ion suppression was evaluated during the experiment. It was found that when mobile phase consists of acetonitrile–water (90:10), the spiked sample demonstrated good linearity between 1 and 100 ng mL^{-1} for PNT. Under the present chromatographic conditions, the run time of each sample was 4.0 min. The retention times were 1.37 and 1.38 min for PNT and IS, respectively.

3.3. Method validation

3.3.1. Selectivity

Selectivity was assessed by comparing the chromatograms of six different batches of blank urine samples (unspiked urine) with the corresponding spiked urine sample. Fig. 3 shows the typical chromatograms of a blank, a spiked urine sample with PNT and IS. There was no significant interferences or ion suppression from endogenous substances observed at the retention times of the analytes (see Fig. 4).

3.4. Linearity of calibration curves and lower limit of quantification

Urine samples were quantified using ratio of the peak area of PNT to that of IS as the assay parameter. Peak area ratios were plotted against PNT concentrations and standard curves

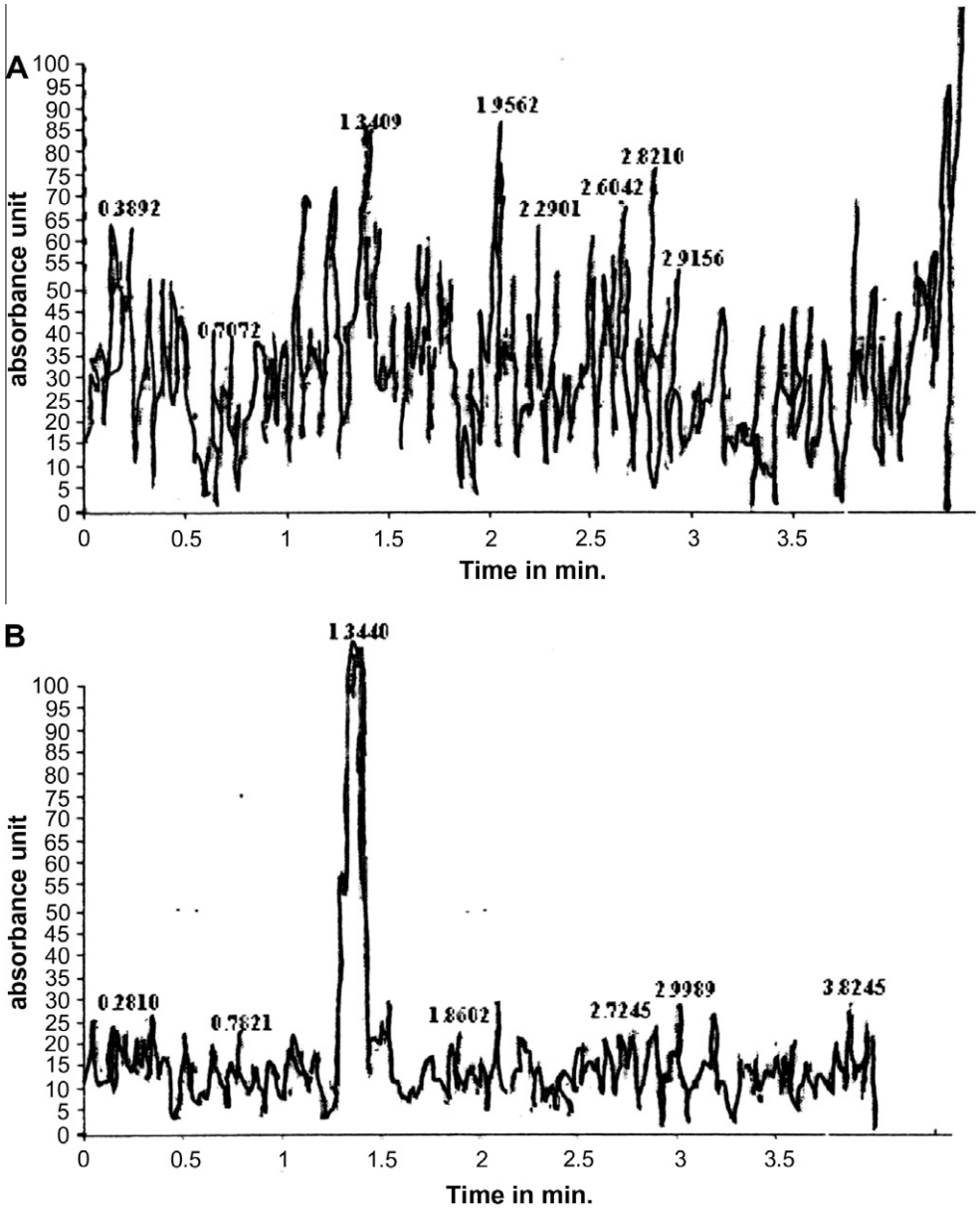

Figure 3 Representative blank chromatogram for pantoprazole (A) and IS (B).

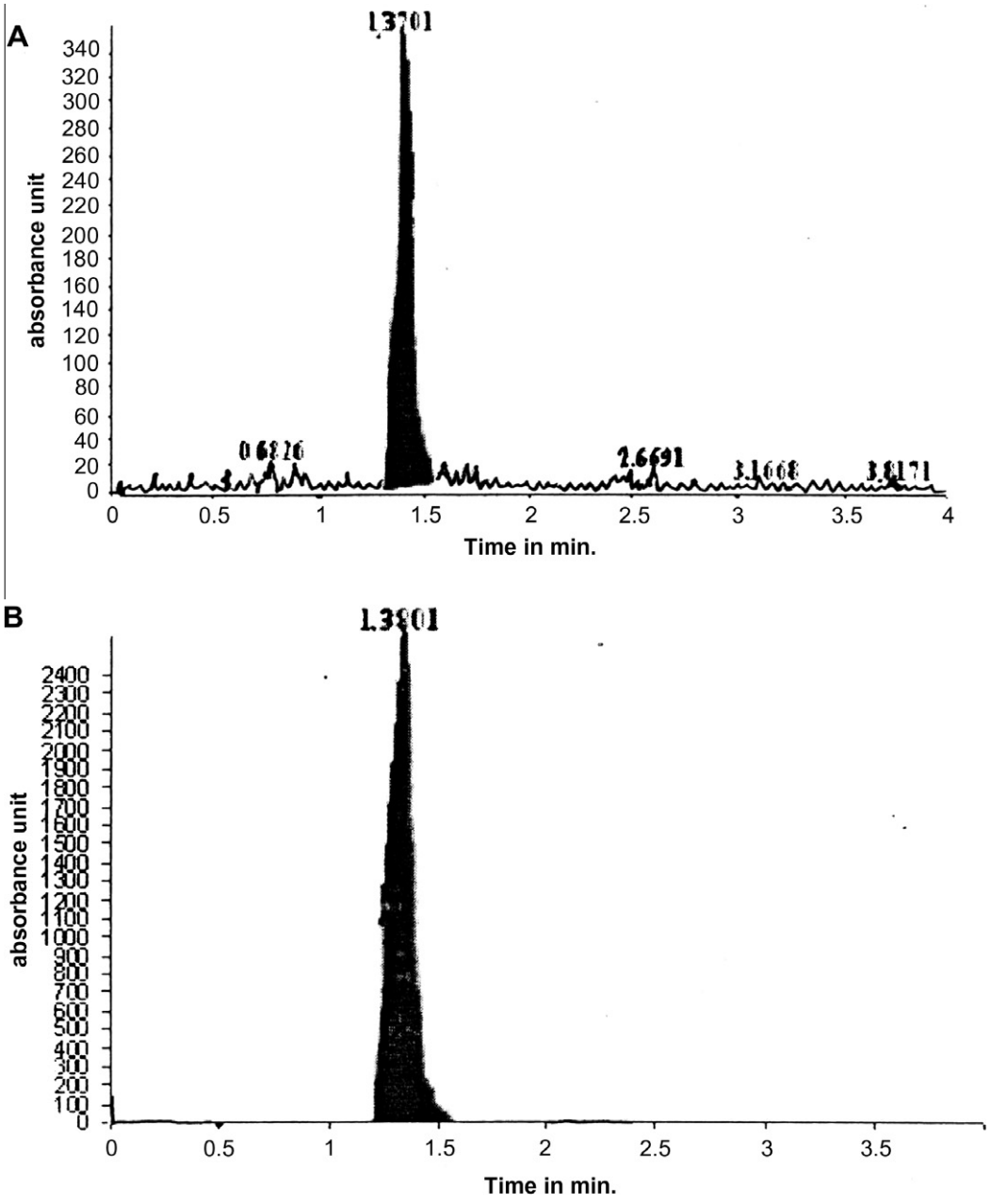

Figure 4 Representative chromatogram of pantoprazole (1.0 ng mL^{-1}) and IS (500 ng mL^{-1}).

in the form of $Y = A + BX$ were calculated using weighed ($1/x^2$) least squares linear regression.

To evaluate linearity, urine calibration curves were prepared and assayed in duplicate on three separate days.

Visual inspection of the plotted duplicate calibration curves and correlation coefficients > 0.99 confirmed that the calibration curves were linear over the concentration ranges of 1–100 ng mL^{-1} for the analyte.

The lower limit of quantification was defined as the lowest concentration on the calibration curve for which the acceptable accuracy of $\pm 15\%$ and a precision below $\pm 15\%$ were obtained. The present LC–MS–MS method offered an LLOQ of 1 ng mL^{-1} in 1 mL of urine sample.

3.5. Accuracy, intra-day and inter-day precision

The accuracy and precision were also assessed by determining QC samples at three concentration levels on three different validation days. The accuracy was expressed by (mean observed concentration)/(spiked concentration) $\times$ 100% and the precision by relative standard deviation (RSD).

Table 1 summarizes the precision and accuracy for the PNT evaluated by assaying the QC samples. The intra-day and inter-day precision were established by performing analyses over a period of 5 days on solutions prepared afresh each day.

Table 1 Accuracy and intra-day precision.

PNT taken ($ng\,mL^{-1}$)	PNT found* ($ng\,mL^{-1}$)	Intra-day RSD (%)	Inter-day RSD (%)	RE (%)
8	7.9	7.0	10.5	1.25
50	49.8	8.6	8.6	0.40
85	84.3	7.4	4.3	0.82

RE, relative error; RSD, relative standard deviation.
* Mean value of seven determinations.

3.6. Recovery study

Absolute recoveries of PNT at three QC levels were determined by assaying the samples as described above and comparing the peak areas of both PNT and IS with those obtained from direct injection of the compounds dissolved in the blank urine.

The recovery of PNT, determined at three concentrations (8, 50, and 85 $ng\,mL^{-1}$) were 84.4 ± 5.4%, 83.4 ± 6.4% and 79.4 ± 4.4% ($n = 6$) respectively.

4. Conclusions

A sensitive LC–MS–MS method for the quantification of pantoprazole in urine sample was developed and validated. The method is rapid, sensitive and highly selective with a LOQ of 1 $ng\,mL^{-1}$. The determination of one urine sample needs 4 min. These results indicated that it is suitable for routine analysis of large batches of biological samples.

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