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Determination of cisapride, its oxidation product, propyl and butyl parabens in pharmaceutical dosage form by reversed-phase liquid chromatography

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Abstract

A simple, rapid and reproducible high-performance liquid chromatography (HPLC) assay for cisapride, its oxidation product (OP), propyl and butyl parabens in a pharmaceutical formulation is described. Chromatography was performed at room temperature by pumping acetonitrile–20 mM phosphate buffer pH 7 (50:50, v/v) at 1.5 ml min^{-1} through C_8 reversed-phase column. Cisapride, OP, propyl and butyl parabens were detected at 276 nm and were eluted at 9.7, 3.1, 5.1 and 7.1 min, respectively. Calibration plots were linear ($r > 0.999$) for all compounds from 0.5 to 200 μg ml^{-1} for cisapride and OP and 0.1–200 μg ml^{-1} for propyl and butyl parabens. Detection limits for cisapride, OP, propyl and butyl parabens were 40, 46, 48 and 54 ng ml^{-1}, respectively. Forced degradation investigations showed that cisapride does not undergo degradation under heat, acidic and basic conditions but it was susceptible to oxidation. The proposed method was successfully applied to the assay of cisapride in the presence of preservatives and OP in a commercial suspension.

Keywords: Cisapride; RP-HPLC; Parabens; Degradation

1. Introduction

Cisapride, 4-amino-5-chloro-N-{1-[3-(4-fluoro-phenoxy)-propyl]-3-methoxy-piperidin-4-yl}-2-methoxy-benzamide (Fig. 1) is widely used for the treatment of some gastro-oesophageal diseases such as oesophageal reflux, non ulcer dyspepsia [1]; caused by disordered motility [2–6]. It is believed to facilitate acetylcholine release from the mynetric plexus of the gut and to activate $5HT_4$ receptors in the intestinal wall. Cisapride has been shown to be effective in treating gastro-oesophageal reflux diseases in adults, children and neonates [1,7,8]. Contrary to other structurally similar but older prokinetic drugs (e.g. metoclopramide), cisapride does not affect psychomotor function or induce central depressant adverse effect [9]. Various assay methods have been reported for the determination of cisapride.

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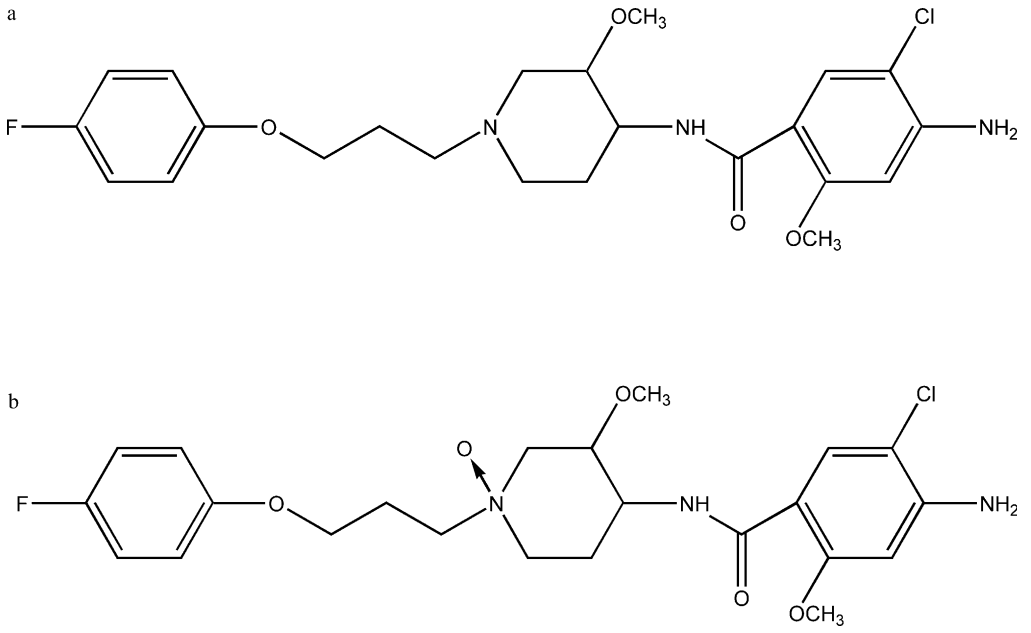

Fig. 1. Chemical structure of cisapride (a) and its OP (b).

In European [10] pharmacopoeia, cisapride has been assayed in bulk drugs using potentiometric titration with 0.1 M perchloric acid. In pharmaceutical preparations, cisapride has been determined by measuring the intrinsic fluorescence of the drug at 355.2 nm with excitation wavelength at 310 nm [11]. Spectrophotometric methods have been reported for the determination of cisapride in pharmaceutical preparations. These included extraction spectrophotometric methods [12,13], reaction with p-dimethylamino cinnamaldehyde and phosphoric acid [14], oxidative coupling with 3-methyl 2-benzothiazolinone (MBTH) or oxidation of the drug with Fe(III) and subsequent chelation of Fe(II) with 1,10-phenantroline or through formation of charge transfer complex with chloranilic acid [15]. A stability indicating HPTLC method has been reported for the assay of cisapride and related impurities in tablets [16]. High performance liquid chromatograph (HPLC) methods have been reported for the determination of cisapride in pharmaceutical preparation [17], plasma [18–23], serum [24,25] or animal tissues [26]. Bioequivalence studies have been performed using reversed phase chromatography [27]. Stereoselective HPLC determination of cisapride in human plasma has been reported and successfully applied to pharmacokinetic studies [28]. Analysis of cisapride in urine was also performed by polarography after nitration to enhance the polarographic response [29].

In the HPLC method developed by Argekar and Sawant [17], a forced degradation study was conducted under acidic and alkaline conditions. They concluded that under the selected conditions, no degradation of cisapride occurs and that the developed method is a stability indicating one. On the other hand, De Condado et al. [25] developed an HPLC method for the analysis of cisapride in serum with electrochemical detection based on the oxidation of cisapride at relatively low potentials. Despite the fact that no information was given on the electrochemical system used (working electrode, reference electrode), evidence of the easiness of cisapride oxidation was nevertheless demonstrated. Under oxidant conditions, cisapride yields the 4-amino-5-chloro-N-{1-[3-(4-fluoro-phenoxy)-propyl]-3-methoxy-1-oxy-piperidin-4-yl}-2-methoxy-benzamide (Fig. 1).

The purpose of this study was to develop a new HPLC method for the determination of cisapride,

its oxidation product (OP), propyl and butyl parabens in a pharmaceutical formulation.

2. Experimental

2.1. Materials

Cisapride and OP were gifted by Janssen Pharmaceutica (Belgium). Propyl and butyl parabens were purchased from Sigma. Acetonitrile, HPLC grade and all other reagents were from Prolabo (France). Sepride (an oral suspension from Laboratoires Simed, Tunisia) labeled to contain 1 mg ml^{-1} cisapride was purchased from a commercial source.

2.2. Apparatus

The HPLC used in this study was a Beckman System Gold Nouveau Model equipped with a pump module (Beckman, Model 125), a variable wavelength UV detector (Beckman, Model 166) and a computer workstation. The parameters used during this study were as follows: UV wavelength: 276 nm; analytical column: Octyl ODS Hypersil, 5 μm, 250 × 4.6 mm I.D. (Beckman); solvents used for the separation were as follows: solvent A, 20 mM orthophosphoric acid adjusted to pH 7 with NaOH; solvant B, acetonitrile. Solvents were delivered isocratically (50:50, v/v). The flow rate was 1.5 ml min^{-1} and the sample injection volume was 20 μl.

2.3. Standard stock solution

A stock standard solution of cisapride, OP, propyl and butyl parabens were prepared in methanol at 500, 4700, 1700 and 1900 μg ml^{-1}, respectively.

2.4. Calibration

Aliquots of standard stock solution of cisapride, OP, propyl and butyl parabens were taken and diluted with methanol to obtain final concentrations of cisapride and OP in the range 0.5–200 μg ml^{-1} while for propyl and butyl parabens dilutions were made to cover a concentration range 0.1–200 μg ml^{-1}. 20 μl of each standard at each level were injected into the chromatograph. Calibration curves were constructed by plotting peak areas against concentration in μg ml^{-1} and the linear relationships was evaluated by calculation of regression lines by the method of least square.

2.5. Sample solution

Bottles were thoroughly mixed before sampling to ensure homogeneity. Suspension equivalent to 1 mg of cisapride (1 ml) was taken in a 50 ml volumetric flask, about 30 ml of methanol was added and the flask was kept in an ultrasonic bath for 10 min, the solution was then diluted to the mark with methanol. After settlement of excipients, 20 μl was taken and injected into the chromatograph.

2.6. Degradation conditions

A stock standard of cisapride was prepared at a concentration of 500 μg ml^{-1} in methanol. For base degradation studies, 200 μl of the cisapride standard was added to 200 μl of 0.1 M NaOH in an eppendorf tube which was sealed to prevent evaporation of solvent during heating. This solution was left at ambient temperature or heated at 80 °C for 4 h. The solution was then neutralized with 200 μl of 0.1 M HCl and diluted by adding appropriate amount of distilled water prior to analysis.

For acid degradation studies, 200 μl of 0.1 M HCl were added to 200 μl of 500 μg ml^{-1} cisapride stock standard in an eppendorf tube, which was sealed. This solution was left at ambient temperature or heated at 80 °C for 4 h. The acidified solutions were neutralized with 200 μl of 0.1 M NaOH and diluted by adding appropriate amount of distilled water prior to analysis.

For oxidative degradation studies, 200 μl of 3% hydrogen peroxide were added to 200 μl of the 500 μg ml^{-1} cisapride stock solution in an eppendorf tube, which was sealed. This solution was left at ambient temperature or heated at 80 °C for 4 h. The solutions was diluted with appropriate amount of distilled water prior to analysis.

For comparison purposes, 200 μl of the cisapride stock standard solution was diluted with 200 μl of distilled water in an eppendorf tube, which was sealed. This solution was left at ambient temperature or heated at 80 °C for 4 h. Solutions was diluted with appropriate amount of distilled water prior to analysis.

A polymerase chain reaction (PCR) temperature programmer (Stuart Scientific, UK) was used for heating samples at required temperatures.

3. Results and discussion

3.1. Method development and optimization

Initial experiments were carried out using mobile phases of phosphate buffer 20 mM, acetonitrile in different proportions and adjusting pH to 2.43. In such conditions, it was not possible to achieve a reasonable separation between cisapride and OP at any mobile phase composition. In an effort to achieve better resolution of the critical pair, cisapride and OP, the pH of the mobile phase was increased. Results showed that pH 7 offers the best pH condition to achieve separation of cisapride and OP. The best compromise between analysis time and resolution of cisapride, OP, propyl and butyl parabens was then 50:50% acetonitrile phosphate buffer 20 mM, pH 7 delivered at a flow rate of 1.5 ml min^{-1}. Under such conditions, the analysis time is 11 min Fig. 2 shows a typical chromatogram of a synthetic mixture of the four compounds.

3.2. Analytical parameters and validation

The optimized method was validated by a standard procedure to evaluate if adequate accuracy, precision, selectivity and linearity has been achieved [30].

Linearity was determined by representing the variation of peak areas with standard concentrations (μg ml^{-1}). Curves were represented by straight lines for all investigated compounds. The correlation coefficients, r values, were found to be significant (cisapride: $t = 151.12$, $P < 0.05$; OP: $t = 130.37$, $P < 0.05$; propyl paraben: $t = 122.88$, $P < 0.05$; butyl paraben: $t = 96.43$, $P < 0.05$) and intercepts was not significantly different from zero (cisapride: $t = -1.4878$, $P > 0.05$; OP: $t = 2.24$, $P > 0.05$; propyl paraben: $t = 1.39$, $P > 0.05$; butyl paraben: $t = 1.03$, $P > 0.05$).

Limit of detection (LOD), is the lowest concentration that can be distinguished from the noise level while LOQ is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision. The LOD and LOQ were calculated using the equation $LOD = A \times S.D./a$, where, S.D. is the standard deviation of the blank and a is the slope of the calibration curve. A was taken as 3 for LOD and 10 for LOQ determinations, respectively. Results of linearity, LOD and LOQ determinations for all analyzed samples are reported in Table 1.

Accuracy is the closeness of the test results obtained by the analytical method to the true value [30]. Accuracy of the developed method was determined using spiked sample solutions, three preparations each, three injections of each preparation.

Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation (R.S.D.) for a statistically significant number of samples [30]. Relative R.S.D. values were calculated for repeated standard injections ($n = 6$) (system precision) as well as repeated injections of multiple sample preparations ($n = 6$) (method precision). The method validation results are shown in Table 2.

3.3. Determination in a pharmaceutical product

In the pharmaceutical formulation containing cisapride, a small amount of OP can be found after long-term storage in the presence of oxygen. In order to demonstrate the ability of this method to detect OP that may appear in the pharmaceutical formulation, a sample of the drug was spiked with a known amount of OP and injected into the chromatograph.

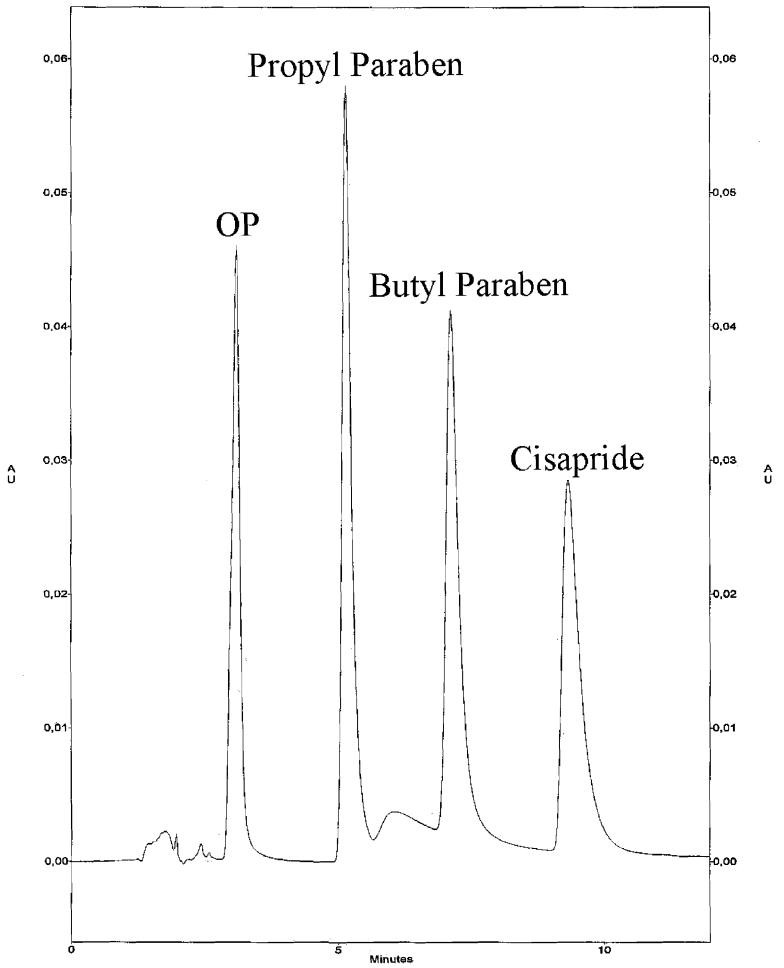

Fig. 2. Chromatogram of a synthetic mixture of OP, propyl paraben, butyl paraben and cisapride. Experimental conditions: phosphate buffer, pH 7; acetonitrile (50:50 v/v), UV detection at 276 nm; flowrate = 1.5 ml min^{-1}.

The chromatogram in Fig. 3 was obtained using the developed HPLC method with a sample of oral suspension (Sepride) spiked with a 19.74 μg of OP. All compounds present in the sample, cisapride, both preservatives are clearly separated. This demonstrates the ability of this method to detect OP in pharmaceutical formulations.

Table 1
Analytical data of the calibration graphs for the determination of cisapride, OP, propyl and butyl parabens by HPLC

Compound	Linearity range (μg ml^{-1})	Slope	Intercept	Correlation coefficient	LOD (ng ml^{-1})	LOQ (ng ml^{-1})
Cisparide	0.5–200	27 626	−24 914	0.9998	40	200
OP	0.5–200	22 047	34 769	0.9997	46	230
Propyl paraben	0.1–200	34 825	36 092	0.9997	48	240
Butyl paraben	0.1–200	30 415	29 806	0.9996	54	310

Table 2
Method validation results

Validation step	Parameter	Cisapride	OP	Propyl paraben	Butyl paraben
System precision	R.S.D. (%)	0.7	1.2	1.83	2.32
Method precision	R.S.D. (%)	0.45	–	1.7	3.76
Accuracy	Spike recovery (%)	99.0	98.5	99.1	98
	Recovery R.S.D. (%)	0.47	0.27	0.42	0.69

3.4. Forced degradation study

HPLC analysis of cisapride submitted to the action of heat, alkaline or acidic conditions did not show any peak other than that of cisapride. The peak area of cisapride in all of those conditions did not show any difference with that of the blank sample. This result is in accordance with the work of Argekar and Sawant [17].

On the other hand, under oxidant conditions, the chromatogram showed the appearance of three peaks. The first at 1.83 min, which is attributed to the remaining hydrogen peroxide, the second at 3.1 min and the peak of cisapride at 9.53 (Fig. 4). The chromatogram of the blank sample showed no peak other than that of cisapride. By using the calibration curve and the peak area of cisapride in the blank solution, the concentration of cisapride was determined, 107.9 μg ml^{-1}. On the other hand, the chromatogram of the sample subjected to ambient temperature oxidation showed a decrease of the peak due to cisapride with appearance of a peak at 3.1 min, which is attributed to OP. The concentration of cisapride and OP were 40 and 103 μg ml^{-1}, respectively. Under such conditions, 63% of the initial cisapride was oxidized to OP.

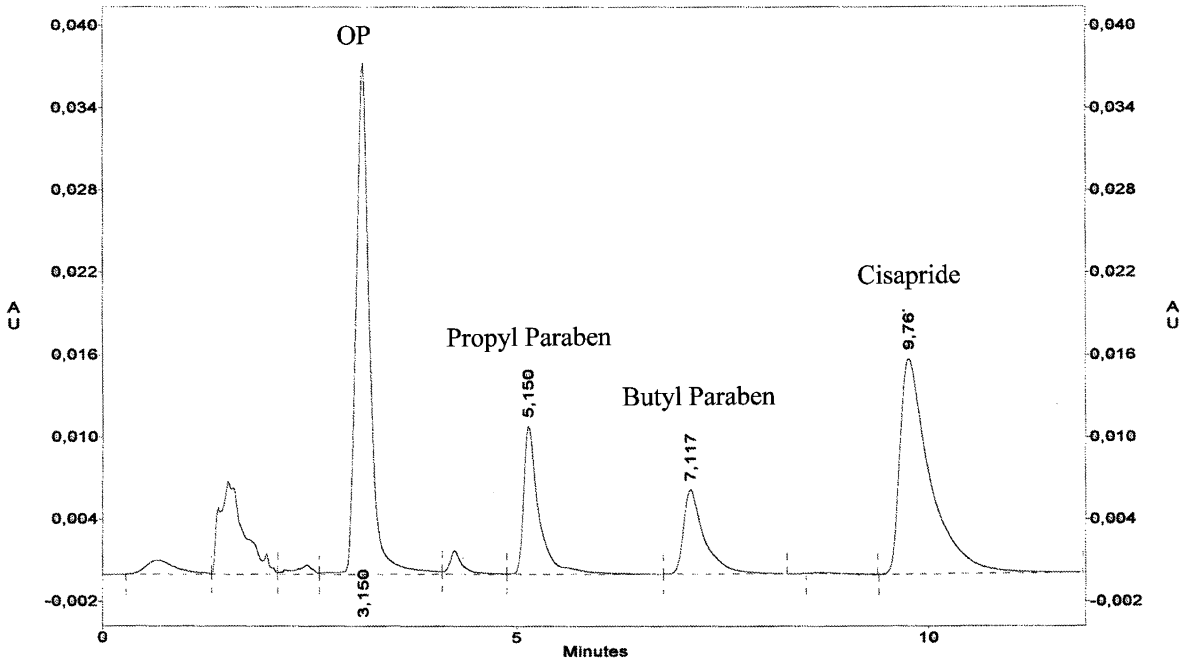

Fig. 3. Chromatogram of diluted sepride solution spiked with 19.74 μg of OP. Experimental conditions: phosphate buffer, pH 7; acetonitrile (50:50 v/v), UV detection at 276 nm; flowrate = 1.5 ml min^{-1}.

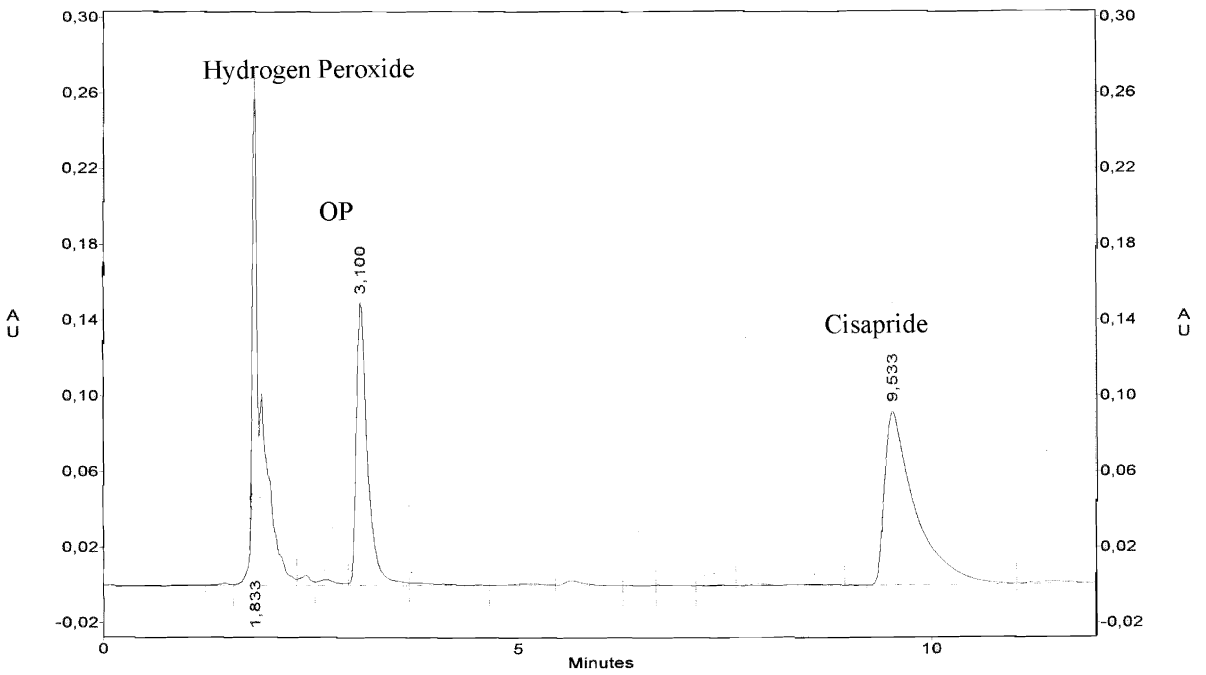

Fig. 4. Chromatogram of cisapride submitted to oxidation with hydrogen peroxide. Experimental conditions: phosphate buffer, pH 7; acetonitrile (50:50 v/v), UV detection at 276 nm; flowrate = 1.5 ml min^{-1}.

Surprisingly, concentration of cisapride submitted to oxidation at 80 °C was higher than at ambient temperature. Cisapride and OP concentrations were 85 and 69 μg ml^{-1}, respectively. Despite the fact that those conditions seems more stressing, only 22% of initial cisapride was oxidized.

In fact, one must keep in mind that hydrogen peroxide is thermodynamically unstable. On the other hand, kinetic of hydrogen peroxide dismutation is too low. Adding a catalyst or heating hydrogen peroxide will provoke an increase of the kinetic of dismutation. This fact explains that the degradation of cisapride is less pronounced if the solution is heated since hydrogen peroxide decomposes before reacting with hydrogen peroxide.

4. Conclusion

The goal of this work was achieved by separating and quantitating cisapride, OP and parabens in oral suspension by HPLC. The concomitant quantitation and analysis in this method provides significant time-saving advantages in pharmaceutical laboratories analysis. HPLC method have been validated for the quantitation of necessary components as shown by the accuracy, linearity, recovery, and precision data.

References

[1] J.E.F. Reynold, K. Parfitt, A.V. Parsons, S.C. Sweetman, Martindale, in: The Extra Pharmacopoeia, 32st ed, Royal Pharmaceutical Society of Great Britain, London, 2002, pp. 879–880.
[2] J.C. Reynolds, P.E. Putnam, Gastroenterol. Clin. North Am. 21 (1992) 567–568.
[3] R.W. Lynda, D. Faulds, Drugs 47 (1994) 116–120.
[4] A. Huttemann, Digestion 34 (1986) 143–147.
[5] H.D. Janish, W. Hautteman, M.H. Bonzo, Hepatogastroenterology 35 (1988) 125–128.
[6] H. Geldof, B. Hazelhoff, M.H. Otten, Aliment Pharmacol. Ther. 7 (1993) 409–415.
[7] F. Onat, B. Yegan, K. Berkman, S. Oktay, Gen. Pharmacol. 25 (1994) 1253–1256.

[8] J.A. Barone, L.N. Jessen, J.L. Colaizzi, R.H. Bierman, Ann. Pharmacother. 28 (1994) 488–493.
[9] R.W. McCallun, C. Prakash, D.M. Campoli-Richards, K.L. Goa, Drugs 36 (1988) 652–681.
[10] European Pharmacopoeia, fourth ed., Council of Europe, Strasbourg, 2002.
[11] M.I. González Martin, C. González Pérez, M.A. Blanco López, Anal. Lett. 27 (1994) 1713–1718.
[12] H.H. Abdine, Alexandrian J. Pharm. Sci. 14 (2000) 75–78.
[13] C.S.P. Sastry, Y. Srivinas, P.V. Subba Rao, Talanta 44 (1997) 517–526.
[14] A.J. Bardhai, K.R. Mahadik, H.N. More, P.D. Panzade, Indian Drugs 36 (1999) 665–666.
[15] C.S.P. Sastry, Y. Srivinas, P.V. Subba Rao, Mikrochim. Acta 126 (1997) 63–67.
[16] T.G. Chandrashekhar, K. Smrita, P.S.N. Rao, S.K. Vyas, C. Dutt, J. Planar Chromatogr. 9 (1996) 138–141.
[17] A.P. Argekar, J.G. Sawant, J. Pharm. Biomed. Anal. 21 (1999) 221–226.
[18] Y. Preechagoon, B.G. Charles, J. Chromatogr. B 670 (1995) 139–143.
[19] S. Cisternino, J. Schlatter, J.L. Saulnier, J. Chromatogr. B 714 (1998) 395–398.
[20] P.B. Rao, S.D. Dhuri, M. Sundaresan, M.A. Dave, Indian Drugs 34 (1997) 505–507.
[21] B.D. Kiss, K.B. Nemes, I. Klebovich, Chromatographia 57 (2003) 47–50.
[22] M.A. Campanero, B. Calahorra, E. Garcia-Quetlas, J. Honorato, J.J. Carballal, Chromatographia 47 (1998) 537–541.
[23] E.S. Park, M. Hahn, Y.S. Rhee, S.C. Chi, Anal. Lett. 34 (2001) 1285–1294.
[24] H.M. Lee, S.J. Choi, C.K. Jeong, Y.S. Kim, K.C. Lee, H.S. Lee, J. Chromatogr. B 727 (1999) 213–217.
[25] M.C. De Condado, A. Malave, M. Dorantes-Hernandez, A. Rathinavelu, J. Off. Anal. Chem. Int. 84 (2001) 9–12.
[26] R. Woestenborghs, W. Lorreyne, F. Van Rompaey, J. Heykants, J. Chromatogr. B 424 (1988) 195–220.
[27] E.K. Lyer, H.P. Tipnis, Indian Drugs 31 (1994) 519–524.
[28] Z. Desta, N.V. Soukhova, A. Morocho, J. Park, S.K. Mahal, D.A. Flockhart, J. Chromatogr. B 744 (2000) 263–272.
[29] M.I. González Martin, C. González Pérez, M.A. Blanco López, Anal. Chim. Acta 368 (1998) 175–181.
[30] G.A. Shabir, J. Chromatogr. A 987 (2003) 57–66.