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Simultaneous estimation of four proton pump inhibitors—lansoprazole, omeprazole, pantoprazole and rabeprazole: development of a novel generic HPLC-UV method and its application to clinical pharmacokinetic study

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ABSTRACT: A highly selective, sensitive and accurate HPLC method has been developed and validated for the estimation of four proton-pump inhibitors (PPI), lansoprazole (LPZ), omeprazole (OPZ), pantoprazole (PPZ) and rabeprazole (RPZ), with 500 μL human plasma using zonisamide as an internal standard (IS). The sample preparation involved simple liquid–liquid extraction of LPZ, OPZ, PPZ and RPZ and IS from human plasma with ethyl acetate. The baseline separation of all the peaks was achieved with 0.1% triethylamine (pH 6.0):acetonitrile (72:28, v/v) at a flow rate of 1 mL/min on a Zorbax C_8 column. The total chromatographic run time was 11.0 min and the simultaneous elution of IS, OPZ, RPZ, PPZ and LPZ occurred at approximately 2.42, 4.45, 5.02 and 9.37 min, respectively. The method was proved to be accurate and precise at linearity range of 20.61–1999.79 ng/mL with a correlation coefficient (*r*) of ≥0.999. The limit of quantitation for each of the PPI studied was 20.61 ng/mL. The intra- and inter-day precision and accuracy values were found to be within the assay variability limits as per the FDA guidelines. The developed assay method was applied to a pharmacokinetic study in human volunteers. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: lansoprazole; omeprazole; pantoprazole; rabeprazole; proton pump inhibitors; human plasma; validation; HPLC; pharmacokinetics

Introduction

Proton pump inhibitors (PPIs), viz. lanoprazole (LPZ), omeprazole (OPZ), pantoprazole (PPZ) and rabeprazole (RPZ), are the first-line treatment for many patients with acid-peptic disorders, including erosive gastro-oesophagal reflux disease (GERD), nonerosive reflux disease (NERD) and duodenal gastric ulcers. PPIs are superior to histamine$_2$-receptor antagonists (ranitidine, cimetidine, famotidine, etc.) in control of gastric acid and in the management of acid-mediated disorders. PPIs selectively and irreversibly inhibit the H^+/K^+-adenosine triphosphatase (ATPase) (proton pump) that performs the final step in the acid secretory process by stimulation of histamine or gastrin or acetylcholine receptors present on the parietal cells. All the PPIs (Fig. 1) are substituted benzimidazole derivatives and they function as prodrugs. They inhibit both basal and stimulated secretion of gastric acid, independently of the nature of parietal cell stimulation (Huber *et al*., 1999; Sachs *et al*., 1995). Following oral administration they are absorbed and enter into the cannalicular lumen of parietal cells and are activated by conversion to the tetracyclic planar sulfenamide. The activated sulfenamide bind covalently to cystine residue on the proton pump, thereby irreversibly inhibiting the H^+/K^+-ATPase and gastric acid secretion (Richardson *et al*., 1998). All PPIs are acid labile, hence orally they are administered as enteric-coated formulations. Following oral administration all PPIs are rapidly absorbed and the maximum concentrations in plasma are attained between 1 and 3 h. Relatively all PPIs have prolonged pharmacodynamic activity (48–72 h), when compared with their short plasma half-life ($t_{½}$) of ~1 h. The maximum concentration in plasma (C_{max}) and area under the time–concentration curve (AUC) does not correlate with acid suppression for all PPIs. All four PPIs are highly protein bound (>95%) and metabolized extensively in liver by CYP2C19 and 3A4 to varying degrees into less active or inactive metabolites and excreted through urine. The oral bioavailabilities of LPZ, OPZ, PPZ and RPZ were found to be 80–85, 30–40, 77 and 52%, respectively. Acid suppression studies comparing OPZ and LPZ, and OPZ and PPZ suggest

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Abbreviations used: ATPase, H^+/K^+-adenosine triphosphatase; GERD, gastro-oesophagal reflux disease; LPZ, lanoprazole; NERD, nonerosive reflux disease; OPZ, omeprazole; PPI, proton pump inhibitors; PPZ, pantoprazole; RPZ, rabeprazole.

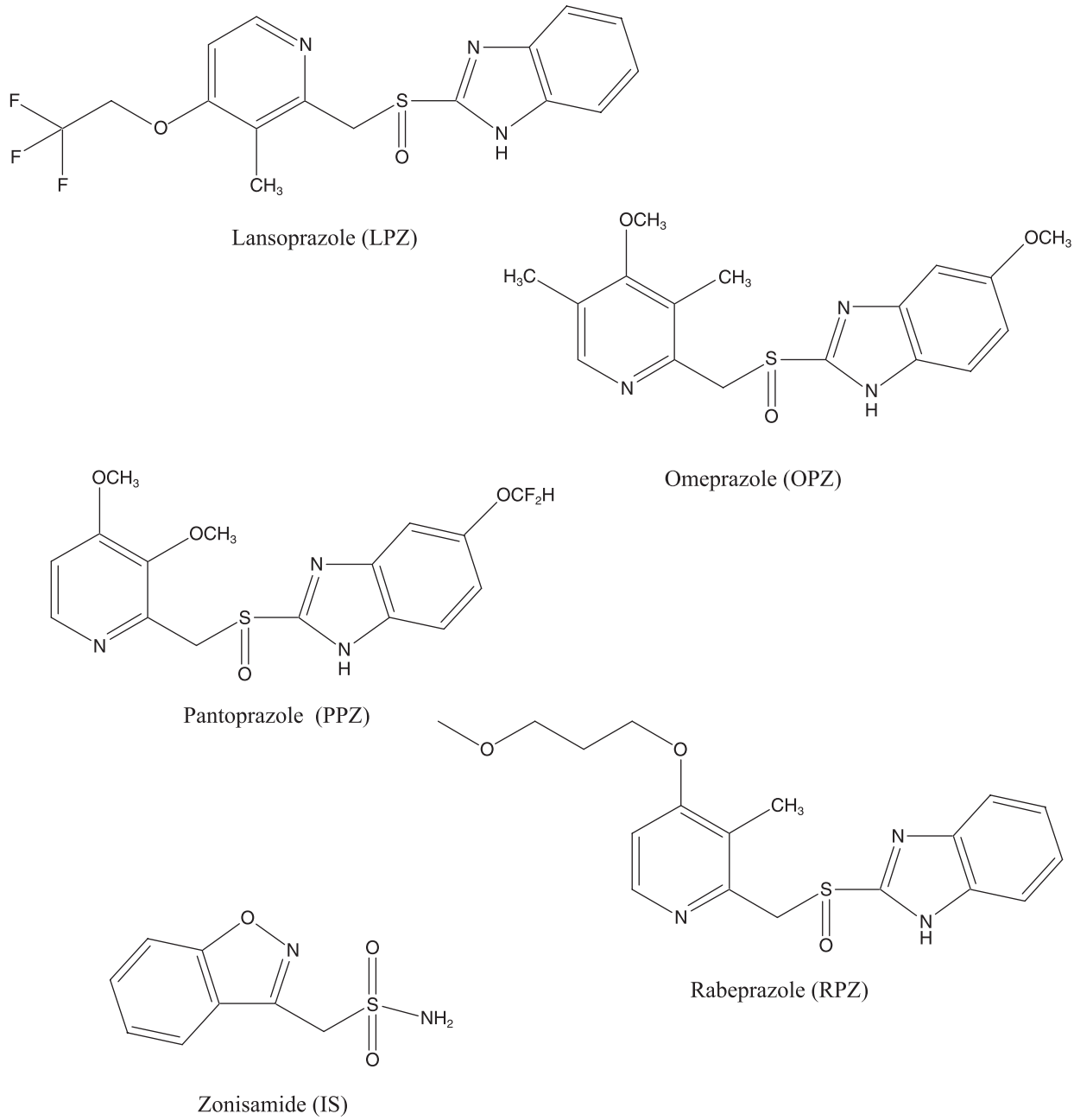

Figure 1. Structural representation of LPZ, OPZ, PPZ, RPZ and IS.

equivalent potency. In other studies, LPZ 30 mg resulted in greater acid suppression than PPZ 40 mg and RPZ gave greater acid suppression than OPZ. Both LPZ and RPZ give more rapid onset of action than other PPIs (Berardi, 2000; Stedman and Barclay, 2000).

For the four PPIs, LPZ, OPZ, PPZ and RPZ, several HPLC methods have been reported for the quantification of each PPI along with their metabolites [LPZ (Aoki *et al*., 1991; Landes *et al*., 1992; Katsuki *et al*., 2001; Miura *et al*., 2004; Uno *et al*., 2005a), OPZ (Mihaly *et al*., 1983; Kobayashi *et al*., 1992; Hofmann *et al*., 2006; Rezk *et al*., 2006; Shimizu *et al*., 2006), PPZ (Xie *et al*., 2005) and RPZ (Uno *et al*., 2005b; Miura *et al*., 2006)]. In 1999, Ekpe and Jacobsen developed a simultaneous method for quantification of LPZ, OPZ and PPZ in order to study the effect of pH and various salts on the stability of the three compounds with no details on validation parameters. Hitherto, there is no method reported in literature for simultaneous quantification of these four PPIs viz. LPZ, OPZ, PPZ and RPZ in biological matrix. In this manuscript, we are presenting a simple HPLC method with UV detection for simultaneous estimation of LPZ, OPZ, PPZ and RPZ with complete validation parameters and application of the newly developed method to a clinical pharmacokinetic study.

Experimental

Chemicals and Reagents

LPZ, OPZ, PPZ, RPZ and IS (Fig. 1) were procured from Formulations Tech, Operations Unit-III of Dr Reddy's Laboratories Ltd, Hyderabad, India. Purity was found to >98.5% for all the compounds. HPLC-grade acetonitrile and analytical-grade *ortho*-phosphoric acid,

triethylamine and ethylenediaminetetra-aceticacid (EDTA) dipotassium salt were purchased from Qualigens, Mumbai, India. All aqueous solutions including the buffer for the mobile phase were prepared with Milli Q (Millipore, Milford, MA, USA) grade water. The control K_2EDTA human plasma was purchased from Cauvery Diagnostics and Blood Bank, Secunderabad, India.

Instrumentation and Chromatographic Conditions

The HPLC system consisted of a Waters 2695 Alliance (Milford, MA, USA) separation module attached to a Waters® 2996 photodiode array detector. A Zorbax SB, C_{18} column (4.6 × 75 mm, 3.5 μm) maintained at ambient temperature (25 ± 2°C) was used for the analysis. The isocratic mobile phase system consisting of 0.1% triethylamine (pH 6.0 adjusted with *ortho*-phosphoric acid):acetonitrile (72:28, v/v, final pH 7.2) was delivered at a flow-rate of 1.00 mL/min through the column to elute the analytes. The eluate was monitored by the PDA detector set at 270 nm. The data were acquired and processed with Millennum[32] software (version 4).

Preparation of Stock and Standard Solutions

Primary stock solutions of LPZ, OPZ, PPZ and RPZ for preparation of calibration standard (CC) and quality control (QC) samples were prepared from separate weighings. The primary stock solutions (1.00 mg/mL) of these analytes and IS were prepared in methanol with 1% ammonia and stored at −20 ± 2°C for 30 days (data not shown). Appropriate dilutions were made in methanol for LPZ, OPZ, PPZ and RPZ to produce the four-in-one working stock solutions of 2.06, 4.12, 10.57, 30.20, 99.98, 151.02, 180.19 and 199.98 μg/mL and on the day of analysis this set of stocks was used to prepare standards for calibration curve (CC). Another set of working stock solutions of LPZ, OPZ, PPZ and RPZ was made in methanol (from second primary stock) at 2.06, 5.98, 90.53 and 170.81 μg/mL for preparation of QC samples. Working stock solutions were stored at approximately 5°C and found to be stable for 15 days (data not shown). Individually QC and CC working stock solutions of FQs were made before spiking into QC and CC plasma samples accordingly. A working stock solution of IS (80 μg/mL) was prepared in methanol from primary stock solution. Calibration standards were prepared by spiking in 490 μL of control human plasma with the appropriate amount of composite stock of LPZ, OPZ, PPZ and RPZ (10 μL) and IS (50 μL) on the day of analysis. Samples for the determination of recovery, precision and accuracy were prepared by spiking control human plasma in bulk at appropriate concentrations [20.61 ng/mL (lower limit of quantitation, LLOQ), 59.75 ng/mL (quality control, QC, low), 905.32 ng/mL (QC medium) and 1708.15 ng/mL (QC high)] and 500 μL volumes were aliquoted into different tubes and stored at −80 ± 10°C until analysis.

Recovery

The recovery of all four analytes, LPZ, OPZ, PPZ and RPZ, along with IS, through liquid–liquid extraction procedure was determined by comparing the responses of the analytes extracted from replicate QC samples (n = 6) with the response of analytes from aqueous standard samples at equivalent concentrations. Recoveries of LPZ, OPZ, PPZ and RPZ were determined at QC low, medium and high concentrations, 59.75, 905.32 and 1708.15 ng/mL, whereas the recovery of the IS was determined at a single concentration of 80 μg/mL.

Sample Preparation

A simple liquid–liquid extraction method was followed for extraction of LPZ, OPZ, PPZ and RPZ from human plasma. To a 500 μL plasma aliquot, 50 μL IS solution (80 μg/mL) and 50 μL 0.2 M NaOH were added and mixed for 30 s on a cyclomixer (Remi Instruments, Mumbai, India), followed by extraction with 2.50 mL of ethyl acetate. The mixture was vortexed for 5 min, followed by centrifugation for 5 min at 4500 rpm on Biofuge at 5°C (Heraus, Germany). The organic layer (2.2 mL) was separated and evaporated to dryness at 40°C using a gentle stream of nitrogen (Turbovap® LV evaporator, Zymark® Kopkinton, MA, USA). The residue was reconstituted in 250 μL of the reconstitution solvent [ammonium acetate buffer (pH 7.5):methanol, 1:1, v/v] and 20 μL was injected onto analytical column.

Validation Procedures

A full validation according to the FDA guidelines (US DHHS, FDA, CDER, 2001) was performed for the assay in human plasma.

Specificity and selectivity. The specificity of the method was evaluated by analyzing human plasma samples from at least six different sources to investigate the potential interferences at the LC peak region for analyte and IS.

Calibration curve. The calibration curve was acquired by plotting the ratio of sum of peak area of each PPI to that of IS against the nominal concentration of calibration standards. The final concentrations of calibration standards obtained for plotting the calibration curve were 20.61, 41.23, 105.72, 302.05, 999.89, 1510.26, 1801.89 and 1999.79 ng/mL. The results were fitted to linear regression analysis without using a weighting factor. The calibration curve for each PPI had a correlation coefficient (r) of 0.999 or better. The acceptance criterion for each back-calculated standard concentration was ±15% deviation from the nominal value except at LLOQ, which was set at ±20% (US DHHS, FDA, CDER, 2001).

Precision and accuracy. The intra-day assay precision and accuracy were estimated by analyzing six replicates at four different QC levels, i.e. 20.61, 59.75, 905.32 and 1708.15 ng/mL. The inter-assay precision was determined by analyzing the four levels of QC samples on three different runs. The criteria for acceptability of the data included accuracy within ±15% standard deviation (SD) from the nominal values and a precision of within ±15% relative standard deviation (RSD), except for LLOQ, where it should not exceed ±20% of accuracy as well as precision (US DHHS, FDA, CDER, 2001).

Stability experiments. The stability of LPZ, OPZ, PPZ, RPZ and IS in the injection solvent was determined periodically by injecting replicate preparations of processed samples up to 48 h (auto-sampler) after the initial injection. The peak-areas of the each analyte and IS obtained at 0 h on day 1 were used as the reference to determine the relative stability of the analyte at subsequent points. In all stability studies two QC concentrations were used, QC low and QC high. Stability of the four PPIs, LPZ, OPZ, PPZ and RPZ, in the biomatrix during 12 h exposure at room temperature in human plasma (bench-top) was determined at ambient temperature (25 ± 1°C) in six replicates at each concentration. The freezer stability of each PI in human plasma was

assessed by analyzing the QC samples stored at −80 ± 10°C for at least 30 days. The stability of LPZ, OPZ, PPZ and RPZ in human plasma following repeated freeze–thaw cycles was assessed using QC samples spiked with LPZ, OPZ, PPZ and RPZ. The samples were stored at −80 ± 10°C between freeze–thaw cycles. The stability of LPZ, OPZ, PPZ and RPZ was assessed after the third freeze–thaw cycle. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (i.e. ±15% SD) and precision (i.e. ±15% RSD).

Pharmacokinetic Study in Humans

Pharmacokinetic studies were performed in healthy male subjects (n = 3). The ethics committee approved the protocol and the volunteers provided written informed consent. Blood samples were obtained following oral administration of LPZ (30 mg tablet, Prevacid), OPZ (20 mg tablet, Losec), PPZ (20 mg tablet, Protium) and RPZ (20 mg tablet, Pariet) to different group of subjects into polypropylene tubes containing EDTA solution as an anti-coagulant at pre-determined time points based on the T_{max} (time to reach maximum plasma concentration in plasma) for each PPI. Plasma was harvested by centrifuging the blood using Biofuge (Hereaus, Germany) at 3500 rpm for 5 min and stored frozen at −80 ± 10°C until analysis.

An aliquot of 500 μL of thawed plasma samples were spiked with IS and processed as mentioned in the Sample Preparation section. Along with study samples, QC samples at low, medium and high concentrations were assayed in duplicate and were distributed among unknown samples in the analytical run. The criteria for acceptance of the analytical runs encompassed the following: (i) not more than 33% of the QC samples were greater than ±15% of the nominal concentration; and (ii) not less than 50% at each QC concentration level must meet the acceptance criteria. Plasma concentration–time data of GFC were analyzed by a non-compartmental method using WinNonlin Version 5.1 (Pharsight Corporation, Mountain View, CA, USA).

Results

Optimization of the Experimental Conditions

Preliminary experiments were carried out to optimize the experimental parameters affecting the chromatographic separation of LPZ, OPZ, PPZ and RPZ along with a structurally close IS (zonisamide) in the pre selected LC-column and their detection by UV. In order to detect LPZ, OPZ, PPZ and RPZ simultaneously with good sensitivity, 270 nm was selected as UV_{max}. Based on the literature information it is evident that PPIs need an alkaline pH for their stability during analysis. Hence we have used triethylamine as one of the component in the mobile phase. The feasibility of different mixtures of solvents such as acetonitrile and methanol with different concentrations of triethylamine, along with variable pH range (5.0–7.0) and different flow-rates (in the range of 0.5–1.5 mL/min) were tested for complete chromatographic resolution of LPZ, OPZ, PPZ and RPZ along with IS. It was found that baseline separation of LPZ, OPZ, PPZ, RPZ and IS was achieved on Zorbax SB C_8 column maintained at ambient temperature using an isocratic mixture of 0.1% triethylamine (pH 6.0):acetonitrile (72:82, v/v, final pH 7.2) with 1.00 mL/min flow rate. The total run time was 11 min. We also confirmed that the putative metabolites (LPZ sulfone and 5-hydroxy LPZ of LPZ; OPZ sulfone and 5-hydroxy OPZ of OPZ; PPZ sulfone and PPZ thioether of PPZ and RPZ sulfone and RPZ thioether of RPZ) of each PPI did not interfere the either detection or separation of all four PPIs investigated in this method (data not shown).

Recovery

The results of the comparison of pre-extracted standards vs post-extracted plasma standards were estimated for LPZ, OPZ, PPZ and RPZ at LQC, MQC and HQC, i.e. 20.61, 59.75, 905.32 and 1708.15 ng/mL, respectively. The absolute recovery of each PPI along with recovery of IS was presented in Table 1.

Validation Procedures

Specificity and chromatography. The method demonstrated excellent chromatographic specificity with no endogenous plasma interferences at retention times of peaks of interest as evaluated by chromatograms of blank human plasma and plasma spiked with LPZ, OPZ, PPZ, RPZ and IS. IS, OPZ, RPZ, PPZ and LPZ were well separated with retention times of 2.42, 4.45, 5.02, 5.83 and 9.37 min, respectively. Figure 2 shows a typical overlaid chromatogram for the control human plasma (free of analytes and IS), control human plasma with IS, and human plasma spiked with OPZ, RPZ, PPZ and LPZ at their respective LLOQ (at 20.16 ng/mL concentration). No interfering peaks from endogenous compounds are observed at the retention times of analytes and IS.

Calibration curve. The plasma calibration curve was constructed using eight calibration standards (20.61–1999.79 ng/mL). The calibration standard curve had a reliable reproducibility over the standard concentrations across the calibration range. Calibration curve was prepared by determining the best fit of peak-area ratios (peak area analyte/peak area IS) vs concentration, and fitted to the $y = mx + c$ (1/x). The average regression (n = 3) was found to be ≥0.999. The lowest concentration with the RSD <20% was taken as LLOQ and was found to be 20.61 ng/mL. The percentage accuracy observed for the mean of back-calculated concentrations for three calibration curves was within 92.3–104 (Table 2).

Accuracy and precision. Accuracy and precision data for intra- and inter-day plasma samples are presented in Table 3. The assay

Table 1. Recovery data for PPZ, OPZ, LPZ, RPZ and IS

	LPZ	OPZ	RPZ	IS
LQC	60.26 ± 1.04	66.39 ± 0.97	67.86 ± 0.96	—
MQC	60.45 ± 0.23	64.65 ± 0.75	66.69 ± 0.77	—
HQC	60.06 ± 7.28	69.33 ± 4.00	65.29 ± 4.05	—
80 μg/mL				76.26 ± 1.75

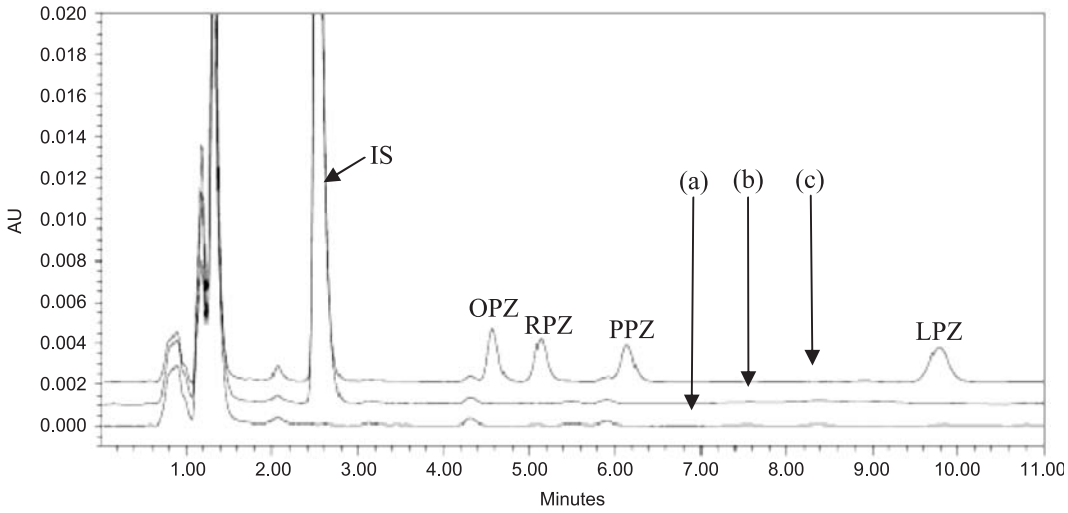

Figure 2. Overlay HPLC chromatograms of a 20 μL injection of an extract from: (a) human blank plasma; (b) human blank plasma spiked with IS; (c) human plasma spiked with OPZ, RPZ, PPZ and LPZ at LLOQ with IS.

Table 2. Linearity data for LPZ, OPZ, PPZ and RPZ

Concentration (ng/mL)	LPZ			OPZ			PPZ			RPZ		
	Mean	CV (%)	Accuracy (%)	Mean	CV (%)	Accuracy (%)	Mean	CV (%)	Accuracy (%)	Mean	CV (%)	Accuracy (%)
CC-1 (20.61)	21.04	2.07	102	20.65	3.04	105	21.04	21.06	96.27	20.58	2.17	99.8
CC-2 (41.23)	38.39	4.79	93.1	38.06	6.09	92.3	38.39	39.53	90.41	40.36	6.07	97.9
CC-3 (105.72)	102.79	7.48	97.2	98.56	3.87	93.2	102.79	102.52	95.59	110.69	7.22	104
CC-4 (302.05)	284.80	1.90	94.2	287.45	0.46	95.1	284.80	283.70	99.17	292.04	1.06	96.6
CC-5 (999.89)	1030.41	2.69	103	1030.54	0.70	103	1030.41	1022.54	98.63	1006.52	1.34	100
CC-6 (1510.26)	1571.15	3.10	104	1558.73	1.64	103	1571.15	1546.19	98.88	1516.72	1.43	100
CC-7 (1801.89)	1891.33	3.20	105	1872.40	0.73	103	1891.33	1855.66	97.61	1795.10	0.59	99.6
CC-8 (1999.79)	2062.79	3.77	103	2039.22	3.00	102	2062.79	2026.29	99.32	1997.62	3.88	99.8

Table 3. Intra- and inter-day precision of determination of PPIs in human plasma

Added concentration (ng/mL)	Measured concentration (ng/mL)											
	LPZ			OPZ			PPZ			RPZ		
	Mean	CV (%)	Accuracy (%)	Mean	CV (%)	Accuracy (%)	Mean	CV (%)	Accuracy (%)	Mean	CV (%)	Accuracy (%)
Intra-day (six replicates at each concentration)												
20.61	20.44	8.81	99.1	20.30	7.74	98.50	21.76	9.35	105.5	21.06	9.52	102.17
59.75	58.27	5.80	97.5	57.85	3.41	96.82	60.94	4.48	102.0	60.67	5.06	101.53
905.32	936.82	2.29	103	930.23	0.99	102.75	925.84	5.69	102.3	911.74	4.03	100.71
1708.15	1780.37	3.30	104	1773.90	2.87	103.85	1755.22	5.51	102.8	1715.31	2.65	100.42
Inter-day (24 replicates at each concentration)												
20.61	19.14	10.78	92.8	21.74	7.25	103	23.08	8.41	112.0	20.22	13.1	98.10
59.75	55.70	4.48	93.2	56.05	1.77	103	61.86	4.12	103.5	61.46	5.48	102.9
905.32	933.39	0.88	103	927.29	0.49	103	925.24	0.81	102.2	893.84	0.76	98.73
1708.15	1762.61	2.66	103	1785.00	1.60	102	1737.42	1.21	101.7	1706.43	1.82	99.90

values on both the occasions (intra- and inter-day) were found to be within the accepted variable limits.

Stability. Over a period of 48 h injection time in the auto-sampler at ambient temperature, over the bench-top for a period of 12 h, following repeated three freeze–thaw cycles −80 ± 10°C and when stored at −80 ± 10°C for 30 days, the predicted concentrations for LPZ, OPZ, PPZ and RPZ at 20.16 and 1999.79 ng/mL deviated within the nominal concentrations. The results were found to be within the assay variability limits (Table 4).

Table 4. Stability data for PPIs quality controls in human plasma

Nominal concentration (ng/mL)		Measured concentration (ng/mL)											
		LPZ			OPZ			PPZ			RPZ		
	Stability	Mean[a]	Accuracy[b] (%)	% CV	Mean[a]	Accuracy[b] (%)	% CV	Mean[a]	Accuracy[b] (%)	% CV	Mean[a]	Accuracy[b] (%)	% CV
LQC (59.75)	0 h (for all)	60.42	101.1	4.43	58.02	97.1	3.83	60.98	102	3.43	61.37	102	5.85
	Third freeze–thaw	62.32	104.3	4.02	58.98	98.7	3.87	63.61	106	3.72	54.74	91.62	2.92
	12 h (bench-top)	61.70	103.3	5.46	55.59	93.0	4.60	62.42	104	2.59	57.32	95.9	5.74
	48 h (in-injector)	61.43	102.8	4.26	56.98	95.3	3.47	63.40	106	8.09	62.96	105	4.48
	Thirty days at −80°C	62.80	105.1	5.81	55.43	92.7	6.07	61.42	102	7.84	61.58	103	4.68
QC (1708.15)	0 h (for all)	1757.48	102.9	0.61	1796.06	105	0.53	1793.64	105	0.92	1693.11	99.1	1.17
	Third freeze–thaw	1755.90	102.8	0.55	1792.14	104	1.32	1798.24	105	2.80	1638.20	95.90	2.72
	12 h (bench-top)	1752.60	102.6	0.54	1801.49	105	0.88	1830.38	107	0.92	1662.59	97.3	1.28
	48 h (in-injector)	1749.49	102.4	0.53	1804.16	105	1.22	1851.39	108	2.37	1641.50	96.1	1.58
	Thirty days at −80°C	1756.54	102.8	0.45	1769.11	103	0.96	1831.24	107	1.90	1564.05	91.5	2.62

[a] Back-calculated plasma concentrations; [b] (mean assayed concentration/mean assayed concentration at 0 h) × 100.

Pharmacokinetic Study in Humans

Figure 3 shows a typical overlaid chromatogram for the control human plasma (free of analyte and IS) and *in vivo* human plasma samples obtained from different pharmacokinetic studies at C_{max} of each PPI, following oral administration of each PPI tablet. The sensitivity and specificity of the assay were found to be sufficient for accurately characterizing the pharmacokinetics of each PPI viz. LPZ, OPZ, PPZ and RPZ. The PK parameters obtained were each PPI are similar to the literature values. Profiles of the mean plasma concentration vs time for each PPI are shown in Fig. 4.

Discussion

Although there have been HPLC methods reported in literature to quantify the investigated PPIs, i.e. LPZ, OPZ, PPZ and RPZ,

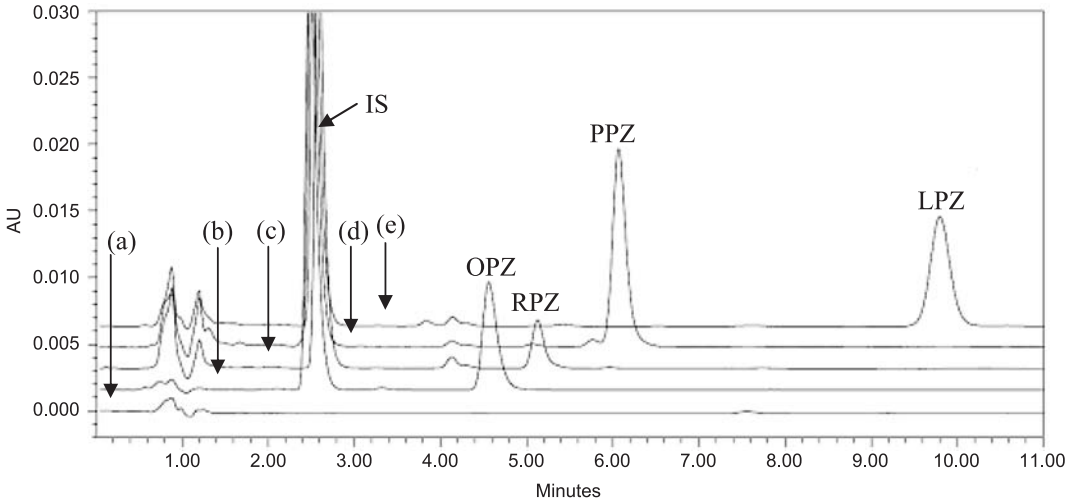

Figure 3. Overlay HPLC chromatograms of a 20 μL injection of an *in vivo* plasma sample obtained from a human volunteer at C_{max} of each PPI following oral dose of each PPI separately: (a) human blank plasma; (b) OPZ; (c) RPZ; (d) PPZ; and (e) LPZ.

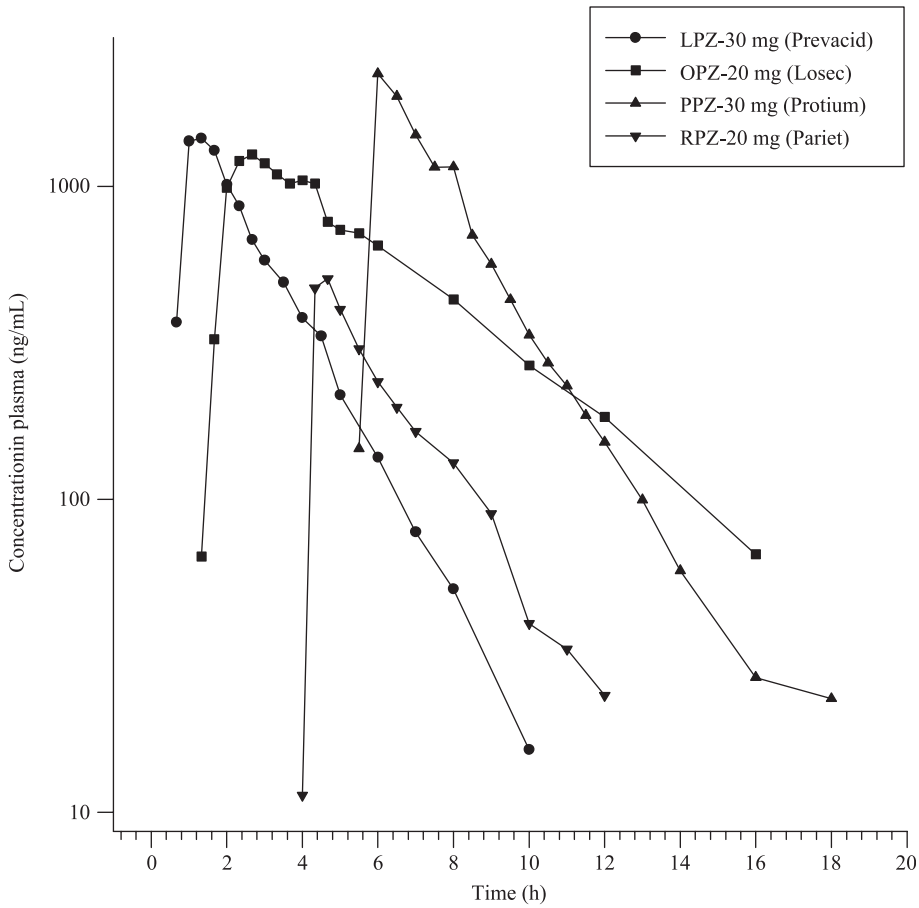

Figure 4. Mean plasma concentration vs time profile for each PPI following oral dosing to human subjects.

individually along with their metabolites [LPZ (Aoki *et al*., 1991; Landes *et al*., 1992; Katsuki *et al*., 2001; Miura *et al*., 2004; Uno *et al*., 2005a), OPZ (Mihaly *et al*., 1983; Kobayashi *et al*., 1992; Hofmann *et al*., 2006; Rezk *et al*., 2006; Shimizu *et al*., 2006), PPZ (Xie *et al*., 2005) and RPZ (Uno *et al*., 2005b; Miura *et al*., 2006)], until now there has been no method reported in literature for simultaneous quantification of these four PPIs viz. LPZ, OPZ, PPZ and RPZ in biological matrix. Ekpe and Jacobsen (1999) have developed a simultaneous method for quantification of LPZ, OPZ and PPZ in order to study the effect of pH and various salts on the stability of the three compounds. We have emphasized the optimization of mobile phase composition and reconstitution solvent in order to maintain the stability of PPIs during bioanalysis.

The generic method, we have developed and validated in human plasma uses a simple liquid-liquid extraction method and achieved the baseline separation of all four PPIs with a run time of 11 min, and we have also demonstrated that the putative metabolites of each PPI did not interfere the either detection or separation of all four PPIs investigated in this method.

Conclusions

In conclusion we have developed and validated a rapid, simple, specific and reproducible HPLC-UV assay to determine four PPIs, i.e. LPZ, OPZ, PPZ and RPZ, simultaneously in human plasma and demonstrated its utility to a clinical pharmacokinetic study.

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