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Short communication

Quantification of oltipraz using liquid chromatography–tandem mass spectrometry and its application to a pharmacokinetic study in rat plasma

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ABSTRACT

An assay method for the determination of oltipraz, a candidate drug for the treatment of liver fibrosis and liver cirrhosis, was developed in rat plasma using a fast-flow protein precipitation (FF-PPT) method coupled with LC–MS/MS for quantification to reduce the labor and to improve the speed of analysis. The applicability of the assay to pharmacokinetic studies was also evaluated. Oltipraz and ethyl-oltipraz, an internal standard (IS), were analyzed by multiple reaction monitoring (MRM) at m/z transitions of 227 → 193 and 241 → 174, respectively. A lower limit of quantification (LLOQ) of 20 ng/mL was observed, with a linear dynamic range from 20 to 4000 ng/mL ($R > 0.997$). The accuracy, precision, dilution, recovery, and stability of the assay were deemed acceptable according to FDA guidelines. Oltipraz concentrations were measured successfully in plasma samples up to 12 h post-dose in rats that had received an oral dose of 60 mg/kg. The findings indicate that the assay method is rapid and sensitive to oltipraz, showing applicability for pharmacokinetics (PK) studies of oltipraz in other small animals, including rats.

1. Introduction

Oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione] has been developed by Rhone-Poulenc (Vitry Cedex, France) for therapeutic applications in the treatment of schistosomiasis and has been studied in terms of pharmacokinetics and toxicity for many years in rats [1]. Recently, the therapeutic effect of oltipraz was reported in rat model with liver cirrhosis induced by N-nitrosodimethylamine [2]. Based on the results, the compound is currently being evaluated in phase II clinical trials in South Korea as a treatment for liver fibrosis and cirrhosis induced by chronic hepatitis, types B and C [3]. Descriptions of several methods for determining concentrations of oltipraz in biological fluids using HPLC have been published [4–6]. However, the retention time of oltipraz tends to be relatively long (~5.8 min) with an overall run time of ~14 min. The amount of eluent required is also relatively high (1.5 mL/min). Some of these reports did not use internal standards, despite extraction steps, and the upper limits of quantification (~500 ng/mL) were not suitable for pharmacokinetic samples. Additionally, not all of the published methods had been characterized in terms of long- and short-term stability, and no report has been published on the quantification of oltipraz with LC–MS/MS, despite the high sensitivity and substantial role of LC–MS/MS in modern analytical methods. The current study describes the development and validation of an analytical assay for the quantitation of oltipraz in rat plasma using FF-PPT [7–9] coupled with LC–MS/MS to increase throughput by shortening processing time and reducing the sample volume requirement according to the guidelines of bioanalytical method validation provided by the FDA [10].

2. Experimental

2.1. Chemicals and reagents

Oltipraz (100% purity) and internal standard (IS) ethyl-oltipraz (99% purity) were supplied by Cheiljedang Corporation (Seoul, Republic of Korea). Acetonitrile (HPLC grade) and formic acid were obtained from Honeywell, Burdick & Jackson (Phillipsburg, NJ, USA) and from Fluka (Cambridge, MA, USA), respectively.

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2.2. Chromatographic and mass spectrometer operating conditions

A HP 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA) consisting of a binary pump, an online degasser, an autosampler, and a column heater was used in the study. A reversed phase Zorbax Eclipse XDB-C18 column (2.1 mm × 100 mm, 3.5 μm; Agilent Technologies) was used for the chromatographic separation of oltipraz and the IS. The mobile phase consisted of acetonitrile–water–formic acid (70:30:0.25, v/v/v) at a flow rate of 0.2 mL/min. The injection volume was 10 μL and the run time was 4 min. Mass spectrometric detection was performed with a Quattro Micro quadrupole mass spectrometer (Waters, Milford, MA, USA) equipped with an electro-spray ionization (ESI) source in the positive-ion mode and operated in multiple reaction monitoring (MRM) mode. The settings of the mass spectrometer for oltipraz and IS were 3.5 kV for the capillary voltage, 30 V for the cone voltage, and 30 V for the collision energy.

2.3. Standards and quality control (QC) samples

A set of oltipraz standard solutions and QC solutions were obtained by successive dilutions of the stock solutions (500 μg/mL for oltipraz and 200 μg/mL for IS) with acetonitrile. A 5-μL aliquot of oltipraz standard solution was spiked into 35 μL of blank rat plasma, resulting in eight non-zero calibration standards (20, 100, 250, 500, 1000, 1500, 3600, and 4000 ng/mL). QC samples with the oltipraz concentrations of 20, 60, 1000 and 3200 ng/mL were prepared in blank plasma. A 40-μL aliquot of thawed plasma or QC samples was transferred to a Whatman Protein Precipitation Unifilter Fast Flow (Whatman, Florham Park, NJ, USA). An aliquot (5 μL) of the working internal standard solution at a concentration of 1000 ng/mL and 200 μL of acetonitrile were dispensed to each well of the filter plate. The Whatman Unifilter was placed on top of a vacuum manifold and a 96-well collection plate was placed at the bottom of the manifold with a reduced pressure (18 in. of Hg). The collection plate was then placed on an autosampler rack and injected into the LC–MS/MS system. All of the samples were covered with aluminum foil or processed under yellow light to minimize the photodegradation of oltipraz.

2.4. Method validation

The selectivity of the analysis was evaluated using six different lots of blank matrices (*i.e.*, samples without oltipraz and IS), zero samples (*i.e.*, blank plasma with the IS), and the lower limit of quantification (LLOQ). Calibration curves were constructed with peak area ratios of oltipraz to internal standard versus nominal concentration of oltipraz in plasma standards ranging from 20 to 4000 ng/mL. Three batches prepared on different days were used to assess the precision and accuracy. The precision was defined as the coefficient of variation at each concentration, and the accuracy was determined by calculating the difference between the calculated and theoretical concentrations. Dilution testing was performed with six replicates of QC samples at a concentration of 16,000 ng/mL, which were then diluted fivefold with blank rat plasma. The relative recovery of oltipraz and IS were determined by comparing mean peak areas of analytes added before extraction into the same six different sources with those of the analytes added to neat solutions at three concentrations of 60, 1000, and 3200 ng/mL. The matrix effect was evaluated as the precision of the peak areas of oltipraz and IS and the peak area ratios (oltipraz/IS) in post-extraction samples. To determine the stability of the stock solution, a set of oltipraz stock solutions was freshly prepared and compared with a stock solution stored for 6 h at 4 °C. Additionally, the stability of oltipraz after three freeze–thaw cycles, post-preparative stability of processed samples in an autosampler, short-term (benchtop) stability, and long-term stability of the analyte in rat plasma were evaluated by analyzing QC samples. Analyte was considered stable if the response of stored samples and fresh samples, or measured analyte concentration and its corresponding theoretical value, differed by less than 15%.

2.5. Application of the assay

Experimental protocols involving animals were reviewed by the Institutional Animal Care and Use Committee (IACUC) of the Korea Institute of Toxicology [11]. To assess the applicability of the assay to pharmacokinetic studies in rats, oltipraz was administrated orally as a suspension in 0.5% sodium carboxymethylcellulose at a dose of 60 mg/kg. Blood samples (approximately 0.25 mL, each) were collected into heparinized tubes via the tail vein at pre-dose (0), 0.25, 0.5, 1, 2, 4, 8 and 12 h post-dose. Plasma was separated by centrifugation (16,100 × *g*, 5 min) and stored at −80 °C until analysis. The plasma concentration versus time data for oltipraz was analyzed with a non-compartmental method using the WinNonlin software (ver. 4.1; Pharsight, Mountain View, CA, USA). The area under the peak corresponding to oltipraz concentration in the plasma-time curve from time zero to the last quantifiable time-point ($AUC_{0-12\,h}$) was calculated by a linear trapezoidal method. The maximum concentration (C_{max}) of oltipraz and the time to reach C_{max} (T_{max}) were obtained directly from the profile of oltipraz concentrations in the plasma [12].

3. Results and discussion

3.1. Mass spectra and chromatography

Preliminary optimization of the collision energy and cone voltage showed that the *m/z* 227 → 193 and 241 → 174 transitions were adequate for the detecting oltipraz and the IS, respectively (Fig. 1). An optimized chromatographic run time of 3.5 min per sample yielded symmetric peaks for both oltipraz and IS with adequate separation for quantitation.

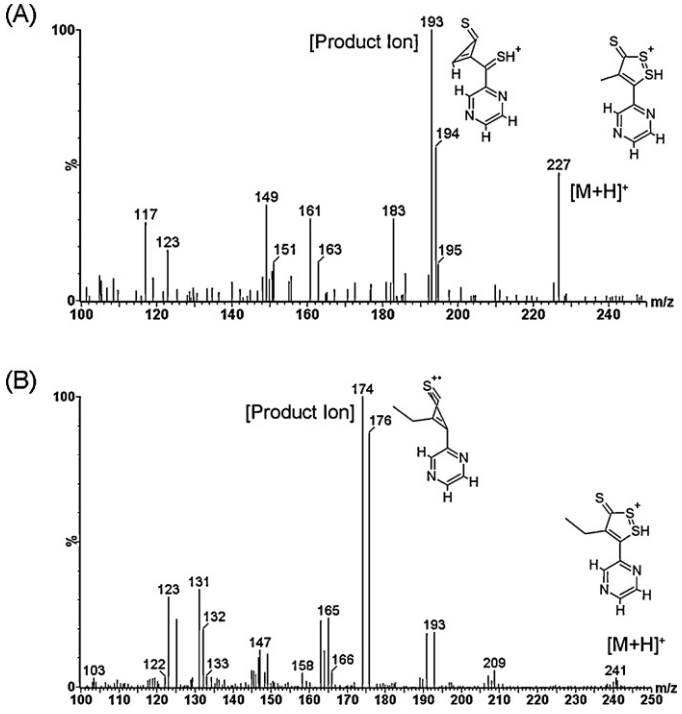

Fig. 1. The structures and product-ion spectra of (A) oltipraz and (B) ethyl-oltipraz, the internal standard.

3.2. Method validation

Chromatograms from six lots of the blank analyses contained no endogenous peaks that co-eluted with any of the analytes or internal standards. The LLOQ was 20 ng/mL with a signal-to-noise ratio greater than 10 and 3.9% of the precision (CV). The calibration curves in rat plasma were linear from 20–4000 ng/mL with a weighting factor of $1/x$ ($y = 4.909x - 11.8$, $r = 0.997$). QC samples were analyzed to determine the intra-day and inter-day accuracy and precision (Table 1). In dilution tests, samples exhibited an accuracy and precision of 1.2% and 7.4%, respectively. The overall process efficiencies for oltipraz analysis were 55.7%, 57.3%, and 51.1% at concentrations of 60, 1000, and 3200 ng/mL, respectively, and the percent recovery for the IS ranged from 40.4 to 50.4%. As a measure of matrix effects, the precision in the peak areas of oltipraz and the IS, and the peak area ratios (oltipraz/IS) in post-extraction samples, were 6.4–8.5%, 4.4–5.6%, and 4.1–5.3%, respectively. Collectively, these data show that the current sample processing conditions yielded adequate recoveries with negligible matrix effects for both oltipraz and the IS. In the stock solution stability of oltipraz and IS, the concentrations in the stored sample were 99.2% and 99.9%, respectively. Benchtop stability, post-preparative stability, long-term storage stability, and three freeze–thaw cycles indicated that oltipraz was stable in rat plasma under the handling and storage conditions used in this study (Table 2).

Table 1
Precision and accuracy for oltipraz in rat plasma.

Batch	Theoretical concentration (ng/mL)			
	LLOQ 20	LQC 60	MQC 1000	HQC 3200
(A) Intra-day ($n = 6$)				
Mean conc.	21.0	59.2	968.3	3275.3
Precision (CV, %)[a]	6.6	1.8	0.9	2.0
Accuracy (RE, %)[b]	5.0	−1.4	−3.2	2.4
(B) Inter-day ($n = 18$)				
Mean conc.		61.6	991.7	3270.9
Precision (CV, %)		4.1	3.0	1.9
Accuracy (RE, %)		2.7	−0.8	2.2

[a] CV (%) = standard deviation of the concentration/mean concentration × 100.
[b] RE (%) = (calculated concentration − theoretical concentration)/theoretical concentration × 100.

Table 2
Stability of oltipraz in quality control samples ($n = 6$).

Batch	Theoretical concentration (ng/mL)		
	60	1000	3200
(A) Benchtop stability at room temperature for 24 h			
Mean conc.	55.9	909.0	2978.7
Precision (CV, %)[a]	3.4	4.3	4.8
Accuracy (RE, %)[b]	−6.8	−9.1	−6.9
(B) Post-preparative stability at 10°C for 2 days			
Mean conc.	66.2	1049.8	3400.9
Precision (CV, %)	2.9	7.3	4.0
Accuracy (RE, %)	10.3	5.0	6.3
(C) Freeze–thaw stability (3 cycles)			
Mean conc.	54.9	872.7	2861.4
Precision (CV, %)	3.5	1.1	2.2
Accuracy (RE, %)	−8.6	−12.7	−10.6
(D) Long-term stability at −80°C for 4 weeks			
Mean conc.	52.5		2837.0
Precision (CV, %)	0.6		3.2
Accuracy (RE, %)	−12.5		−11.3

[a] CV (%) = standard deviation of the concentration/mean concentration × 100.
[b] RE (%) = (calculated concentration − theoretical concentration)/theoretical concentration × 100.

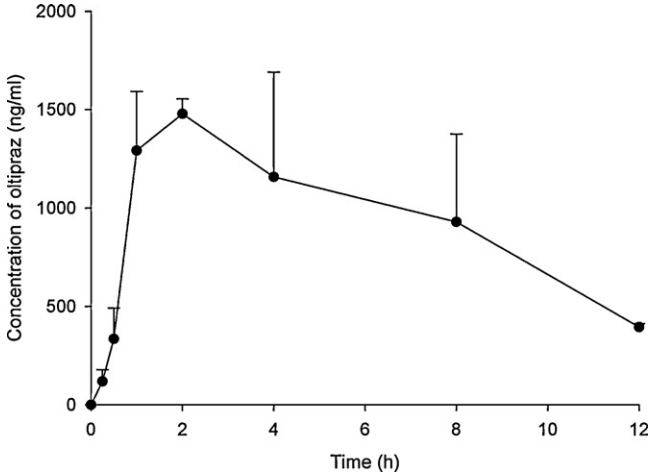

Fig. 2. A temporal profile shows the plasma concentration of oltipraz in rats that had received an oral dose of 60 mg oltipraz/kg (mean ± standard deviation, $n = 3$).

3.3. Applicability to pharmacokinetic studies

Oltipraz concentrations were readily measurable in all plasma samples collected up to 12 h post-dose, indicating that the current assay was adequate for determining the pharmacokinetic characteristics of oltipraz (Fig. 2). The C_{max}, T_{max}, and $AUC_{0-12\,h}$ were 1596.8 ± 135.4 ng/mL, 2.3 ± 1.5 h, and 11330.6 ± 1849.1 ng h/mL, respectively.

4. Conclusions

An analytical assay for determining oltipraz levels in rat plasma was developed and validated in terms of the selectivity, linearity, accuracy, precision, dilution, recovery, matrix effects, and stability. The assay boasts a limit of quantification of 20 ng/mL and a wide linear dynamic range from 20 to 4000 ng/mL, with a correlation coefficient approaching unity. The assay features a simple sample preparation procedure using a fast flow protein precipitation (FF-PPT) method, a relatively short HPLC run time, and the sensitivity and reliability required from pharmacokinetic studies. Thus, this assay may be useful for analyses of oltipraz in the further development of oltipraz as a new drug.

References

[1] M.L. Clapper, Chemopreventive activity of oltipraz, Pharmacol. Ther. 78 (1998) 17–27.
[2] K.W. Kang, Y.G. Kim, M.K. Cho, S.K. Bae, C.W. Kim, M.G. Lee, S.G. Kim, Oltipraz regenerates cirrhotic liver through CCAAT/enhancer binding protein-mediated stellate cell inactivation, FASEB J. 16 (2002) 1988–1990.
[3] C.Y. Ahn, S.K. Bae, S.H. Bae, T. Kim, Y.S. Jung, Y.C. Kim, M.G. Lee, W.G. Shin, Pharmacokinetics of oltipraz in diabetic rats with liver cirrhosis, Br. J. Pharmacol. 156 (2009) 1019–1028.
[4] S.K. Bae, S.C. Bu, E.J. Kim, S.H. Kim, S.G. Kim, M.G. Lee, Determination of a chemopreventive agent, oltipraz, in rat plasma and urine by high-performance liquid chromatography, Res. Commun. Mol. Pathol. Pharmacol. 110 (2001) 133–138.
[5] R.G. Christensen, W. Malone, Determination of oltipraz in serum by high-performance liquid chromatography with optical absorbance and mass spectrometric detection, J. Chromatogr. 584 (1992) 207–212.
[6] J.L. Bennett, J. McMillan, N.V. Dimitrov, High-performance liquid chromatographic method for the analysis of oltipraz in human serum and urine, J. Chromatogr. 573 (1992) 146–149.
[7] C.R. Mallet, Z. Lu, R. Fisk, J.R. Mazzeo, U.D. Neue, Performance of an ultra-low elution-volume 96-well plate: drug discovery and development applications, Rapid Commun. Mass Spectrom. 17 (2003) 163–170.
[8] M.C. Rouan, C. Buffet, F. Marfil, H. Humbert, G. Maurer, Plasma deproteinization by precipitation and filtration in the 96-well format, J. Pharm. Biomed. Anal. 25 (2001) 995–1000.

[9] R.E. Walter, J.A. Cramer, F.L. Tse, Comparison of manual protein precipitation (PPT) versus a new small volume PPT 96-well filter plate to decrease sample preparation time, J. Pharm. Biomed. Anal. 25 (2001) 331–337.
[10] U.S. Department of Health and Human Services, Guidance for Industry: Bioanalytical Method Validation, Center for Drug Evaluation and Research, Food and Drug Administration, 2001.
[11] M. Shalev, APHIS, FDA and NIH issue memorandum of understanding on laboratory animal welfare, Lab. Anim. (NY) 35 (2006), 13-13.
[12] M. Gibaldi, D. Perrier, Pharmacokinetics, 2nd Revised and Expanded, Informa HealthCare, New York, 1982, pp. 409–416.