Journal of Chromatography, 426 (1988) 216–222
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4067

Note

Improved procedure for quantitation of omeprazole and metabolites using reversed-phase high-performance liquid chromatography

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(First received June 25th, 1987; revised manuscript received November 27th, 1987)

Omeprazole, 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulphoxide]-1H-benzimidazole, is currently being investigated by the Digestive Disease Branch of the National Institute of Diabetes, Digestive and Kidney Diseases at the Clinical Center of the National Institutes of Health. Its use as an anti-ulcer agent in upper gastro-intestinal tract ulcers and in the treatment of the Zollinger–Ellison syndrome is being evaluated. It is believed omeprazole inhibits secretion of hydrochloric acid from the parietal cells of the stomach by selectively blocking the activity of the hydrogen–potassium ATPase system [1,2]. This system appears necessary for transport of hydrogen ions into the lumen of the stomach. This selectivity of omeprazole for the H^+/K^+-ATPase system may allow for long-term acid reduction independent of any endogenous stimuli.

A review of the published literature reveals few methods that are currently being employed for the quantitation of omeprazole and its two major metabolites, the sulphone and the sulphide. One such method quantitates these compounds both in the plasma and urine utilizing high-performance liquid chromatography (HPLC) with UV detection [3]. Although this method was able to detect all three compounds at sensitivities of 5, 30 and 15 ng/ml for omeprazole, sulphone and sulphide, respectively, resolution of these components, the peak sharpness (widths) and the plasma extract front seen in the chromatograms did not appear to be supportive of the adequate quality control necessary at these levels. In addition, the coefficients of variation for within-day and day-to-day assays were only reported for concentrations well above their sensitivity values. Another method utilizing normal-phase liquid chromatography and UV detection re-

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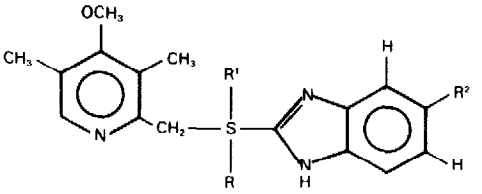

	R	R_1	R_2
Omeprazole	O	–	OCH_3
Sulphone	O	O	OCH_3
Sulphide	–	–	OCH_3
H168/24	O	–	CH_3

Fig. 1. Structures of omeprazole, omeprazole metabolites and internal standard.

ported sensitivities for omeprazole at 10 ng/ml when analyzed alone or in conjunction with the sulphone metabolite [4], however, sensitivities declined by two- to three-fold upon analysis which also included the sulphide metabolite.

We report here a method for simultaneous determination of omeprazole and its two metabolites that has been significantly modified to achieve greater sensitivity with better reproducibility and accuracy.

EXPERIMENTAL

Materials

Omeprazole, omeprazole sulphone, omeprazole sulphide and the internal standard (H168/24) were kindly provided from Hässle (Mölndal, Sweden) (Fig. 1). Spectral-grade dichloromethane, hexane, methanol and acetonitrile (American Scientific Products, American Hospital Supply Corporation; Muskegam, MI, U.S.A.) were utilized along with double-distilled water. Sodium phosphate (dibasic, monobasic, Fisher Scientific, Silver Springs, MD, U.S.A.) were analytical grade. All solvents for HPLC were passed through a 0.45-μm filter (Millipore, Bedford, MA, U.S.A.) and degassed prior to use.

High-performance liquid chromatography

The liquid chromatograph was equipped with an UV detector (Spectroflow Model 783, Kratos Analytical, Ramsey, NJ, U.S.A.) operated at 302 nm and an isocratic solvent delivery system (Beckman Model 110A, Beckman Instruments, Columbia, MD, U.S.A.). The analytical column (Beckman Ultrasphere, San Ramon, CA, U.S.A.), 15 cm $\times$ 4.6 mm I.D., 5 μm particle size, C_8 reversed-phase, was preceded by a 7 cm $\times$ 2.2 mm I.D. guard column (Pell, C_8, 30–40 μm particles; Alltech Assoc., Deerfield, IL, US.A.). The mobile phase consisted of methanol–acetonitrile–0.025 *M* phosphate buffer (40:8:52, v/v). The pH was adjusted to 7.40 with 85% (v/v) phosphoric acid (Fisher Scientific, Silver Spring, MD, U.S.A.). The flow-rate was 1.1 ml/min with a back-pressure of 10–14 MPa and an operating temperature of 25 °C.

Preparation of drug solutions and plasma calibration standards

Stock solutions of each compound were prepared in methanol and subsequent dilutions made in an aqueous carbonate buffer (pH 9.8, 0.1 *M*). Standards were prepared by spiking drug-free pooled human plasma from the appropriate stock

solutions (1 μg/ml in carbonate buffer, pH 9.8, 0.1 *M*) to obtain concentrations ranging from 5 to 200 ng/ml. No degradation of solutions was observed over a period of three months when refrigerated.

Extraction procedure

The following method was utilized in the extraction of the compounds from plasma: internal standard H168/24 (1 μg/ml, 100 μl), dichloromethane (5.0 ml), hexane (5.0 ml) and carbonate buffer (150 μl, pH 9.8, 0.1 *M*) were added to 1.0 ml of plasma in a 15-ml disposable screw-cap culture glass tube (Kimble Culture tubes, 125×16 mm; Scientific, Gaithersburg, MD, U.S.A.). After vortexing for 20 s followed by centrifugation at 1000 *g* for 5 min the tubes were placed in a beaker of methanol in which dry ice had been added to lower the temperature. After freezing of the bottom aqueous phase the organic phase was decanted into another 15-ml glass tube and evaporated under a gentle stream of nitrogen at room temperature. The residue was reconstituted in 250 μl of the mobile phase, vortexed for 30 s and centrifuged for 2 min at 1000 *g*. Depending on the expected sample concentrations injection volumes were adjusted from 15 to 100 μl utilizing an automatic sampler (WISP Model 710B, Waters, Millipore, Milford, MA, U.S.A.).

Quantification and quality control

All compounds were quantitated by comparison of the peak height of the compounds to the internal standard using a calibration curve. The peak-height ratios were plotted against concentrations of each compound and analyzed by linear regression to generate regression parameters of the calibration curves. The lower sensitivity limit of the procedure was determined by assaying spiked plasma samples at concentrations ranging from 1 to 20 ng/ml. The sensitivity limit was then arbitrarily defined as the concentration at which the signal-to-noise ratio was 3.

Inter-day variability was determined by assessing the reproducibility of the standard curves ($n=6$) with respect to both their slopes and peak-height ratios for each concentration (5, 10, 20, 50, 100 and 200 ng/ml). The intra-day variability was assessed by performing a replicate ($n=5$) analysis using plasma samples containing 10.0 and 125 ng/ml for each compound. Aqueous solutions containing known amounts of each compound were compared with spiked plasma standards undergoing analysis to calculate the percentage recovery at various concentrations. Basic statistical parameters, e.g. means, standard deviation, correlation and variance coefficients and linear regression, were computed by utilizing standard programs available in a statistical software package of a Hewlett-Packard 85 microcomputer (Hewlett-Packard Model 85, Hewlett-Packard, Corvallis, OR, U.S.A.).

RESULTS AND DISCUSSION

Representative chromatograms are presented in Fig. 2 of assayed samples of blank human plasma, aqueous buffer spiked with omeprazole, sulphone and sul-

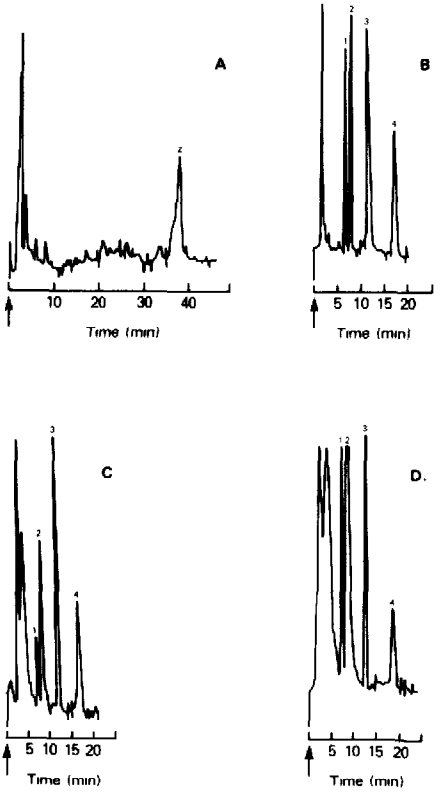

Fig. 2. Chromatogram of (A) a blank plasma extract, (B) a spiked aqueous buffer solution (omeprazole, sulphone, sulphide concentration 100 ng/ml, internal standard concentration 500 ng/ml), (C) an extract of control human plasma (omeprazole, sulphone, sulphide concentration 20 ng/ml) and (D) an extract of a plasma sample from a patient. Peaks: 1 = omeprazole sulphone; 2 = omeprazole; 3 = internal standard, H168/24; 4 = omeprazole sulphide. Chart speed 0.1 cm/min, range 0.005, injection volume = 100 μl.

phide, control human plasma and a patient plasma sample. The chromatogram of blank plasma reveals a small endogenous peak with a similar retention time to that of omeprazole, however, this peak was generally absent depending on the individual plasma samples. There was an additional endogenous peak which was eluted at 39 min, but, with a carefully timed injection sequence, could be eluted earlier thereby allowing for shorter run times. In the plasma containing the compounds the eluted peaks are clean, sharp and symmetric. Retention times (relative retention times in parentheses) for sulphone, omeprazole, internal standard and the sulphide were 7.5 min (0.57), 8.8 min (0.67), 13.0 min (1.0) and 19.7 min (1.5), respectively.

The standard calibration curves constructed from runs of spiked plasma standards were linear and highly reproducible. The mean slopes ($n = 6$) studied over a two-month period were 0.0204 (coefficient of variation, C.V., 4.7%), 0.0058 (C.V. 5.0%) and 0.0136 (C.V. 7.5%) for omeprazole, sulphone and sulphide, respectively. The correlation coefficients determined from linear regression of peak

TABLE I

INTER-DAY VARIABILITY/ACCURACY DATA COLLECTED OVER A TWO-MONTH PERIOD

Compound	Concentration injected (ng/ml)	Observed concentration (mean ± S.D.) (ng/ml)	C.V. (%)
Omeprazole	7.5	7.7 ± 1.1	14.1
	125.0	120.8 ± 6.7	5.4
Sulphone	7.5	8.2 ± 1.0	12.5
	125.0	126.0 ± 8.1	6.4
Sulphide	7.5	7.4 ± 0.4	5.3
	125.0	128.1 ± 5.6	4.4

TABLE II

REPRODUCIBILITY DATA FOR SIX STANDARD CURVES OBTAINED OVER A TWO-MONTH PERIOD IN PLASMA

Compound	Spiked concentration (ng/ml)	Observed peak-height ratios (mean ± S.D.)	C.V. (%)
Omeprazole	5.0	0.120 ± 0.008	6.8
	10.0	0.210 ± 0.015	7.2
	20.0	0.400 ± 0.017	4.3
	50.0	0.960 ± 0.044	4.6
	100.0	2.000 ± 0.059	3.0
	200.0	4.100 ± 0.207	4.9
Sulphone	10.0	0.057 ± 0.008	13.9
	20.0	0.129 ± 0.013	9.7
	50.0	0.280 ± 0.033	11.8
	100.0	0.585 ± 0.034	5.8
	200.0	1.190 ± 0.038	3.2
Sulphide	10.0	0.140 ± 0.011	8.0
	20.0	0.253 ± 0.021	8.1
	50.0	0.670 ± 0.054	8.0
	100.0	1.368 ± 0.110	8.1
	200.0	2.710 ± 0.215	7.9

heights of each compound versus concentration were never less than 0.995. Two quality assurance samples ($n=6$) spiked with 7.5 and 125.0 ng/ml of each compound were simultaneously analyzed with the standard curves. Compounds generally administered to ulcer patients such as the H_2-receptor antagonists were analyzed for possible interference but none coeluted with the drug or metabolites.

Table II lists the results of the inter-day variability (mean, S.D., C.V.) from the six runs at each concentration measured. Intra-day variability of the method determined from plasma standards containing 10.0 and 125 ng/ml for each compound were as follows: at 10.0 ng/ml the values were 2.7, 10.3 and 2.6% and at

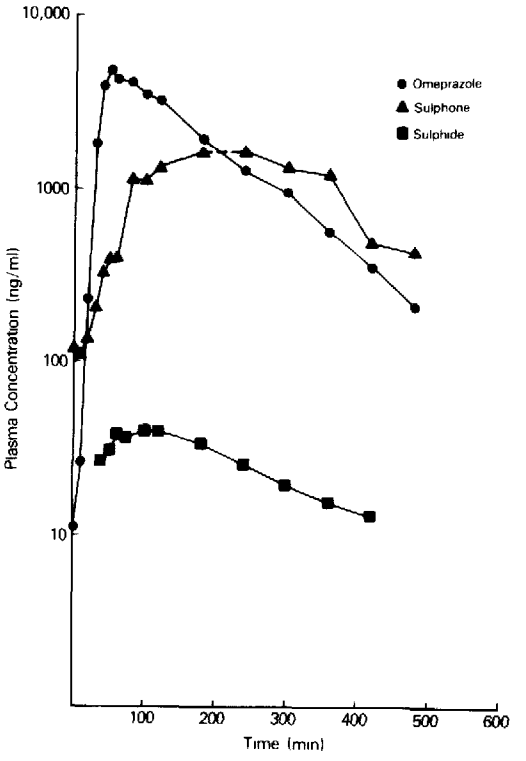

Fig. 3. Plasma levels of patient on a daily oral dose of omeprazole.

125 ng/ml were 2.0, 2.7 and 3.5% for omeprazole, sulphone and sulphide, respectively. The sensitivity of the assay for omeprazole, sulphone and sulphide was at least 5, 10 and 7.5 ng/ml, respectively.

Repeated attempts at reproducing the reported extraction procedure of a single extraction with methylene chloride [3] invariably resulted in unacceptably large interfering peaks which led to a greater than 30% variation in terms of accuracy, therefore we elected to modify the extraction procedure. The recovery computations were based on direct injection of stable buffered (pH 9.8) solutions of each compound onto the HPLC system. The absolute recovery of each compound following the extraction procedure was omeprazole 96% (C.V. 8.4%), sulphone 42% (C.V. 4.28%) and sulphide 96% (C.V. 1.88%). Although the recovery for sulphone was low, adequate sensitivity was still achieved.

The pharmacokinetics of omeprazole has been presented in studies utilizing various dosage forms [5]. Graphical representation of the plasma concentration versus time profile for a patient given 80 mg orally of enteric-coated granules in hard gelatin capsules is presented in Fig. 3. It is noted that all three compounds were measurable including the sulphide metabolite with the latter determined at levels unobtainable in other methods [3,4]. In subsequent patients, timed samples were obtained for 24 h so as to better characterizing the pharmacokinetic profile.

In conclusion the HPLC method presented for plasma determination of omeprazole and its two metabolities is not only sensitive and selective but in addition is markedly improved in both its accuracy and reproducibility when compared to earlier methods.

ACKNOWLEDGEMENTS

We thank Drs. R.T. Jensen, P.N. Maton, R. Vinayek and J.R. Minor for their clinical support, Ray Greene for technical support, and DeAnna Daniels for her secretarial skills.

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