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DETERMINATION OF OMEPRAZOLE AND METABOLITES IN PLASMA AND URINE BY LIQUID CHROMATOGRAPHY

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SUMMARY

Omeprazole, a substituted benzimidazole and a new gastric acid inhibitor, has been determined in plasma and urine, together with three of its metabolites — the sulphide, the sulphone and the hydroxy compound. The methods comprise extraction from the biological materials with methylene chloride, followed either by direct injection of the extract onto a normal-phase liquid chromatography column or evaporation, dissolution and injection onto a reversed-phase system. The compounds were detected using ultraviolet spectrometry. The absolute recoveries obtained were mostly above 95%. The minimum determinable concentration for omeprazole was 20 nmol/l in plasma (relative standard deviation 10—15%) and 50 nmol/l in urine. The metabolites could also be determined at the same levels.

INTRODUCTION

Studies both in vitro [1, 2] and in vivo [3, 4] have shown that omeprazole, 5-methoxy-2-{[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl}-1H-benzimidazole (Fig. 1, I), is a potent inhibitor of gastric acid secretion. The mechanism of omeprazole is different from that of H_2-receptor antagonists and anticholinergic agents and it functions by direct interaction with the proposed gastric proton pump, the H^+, K^+-ATPase [5]. This may be a highly selective clinical means of suppressing the acid secretory process. The effect of omeprazole in dog and in man has been shown not to be correlated to the peak concentration in plasma but to the area under the plasma curve. Since a large number of blood plasma samples have continuously been generated in the documentation of this new drug, a simple method to determine omeprazole in plasma was required to monitor these samples.

The metabolism of omeprazole has been studied by Hoffmann et al. [6]. This present paper describes methods for the determination of omeprazole and three

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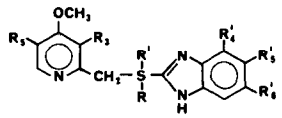

	R_3	R_5	R	R'	R_4'	R_5'	R_6'
Omeprazole(I)	CH_3	CH_3	O	-	H	OCH_3	H
Sulphone (II)	CH_3	CH_3	O	O	H	OCH_3	H
Sulphide (III)	CH_3	CH_3	-	-	H	OCH_3	H
Hydroxyomeprazole (IV)	CH_3	CH_2OH	O	-	H	OCH_3	H
H 168/24(V)	CH_3	CH_3	O	-	H	CH_3	H
H 153/52(VI)	H	H	O	-	CH_3	H	CH_3

Fig. 1. Chemical structures of omeprazole, metabolites and internal standards.

of its metabolites (Fig. 1) — the sulphone (II) and the sulphide (III) in plasma, and the hydroxy metabolite (IV) in plasma and urine. The methods comprise extraction from plasma into methylene chloride followed either by direct injection of part of the organic extract onto a normal-phase liquid chromatography (LC) column or, for the more polar hydroxy metabolite, evaporation of the organic extract, dissolution into an aqueous phase and injection onto a reversed-phase column. The compounds are detected by an ultraviolet (UV) monitor.

Using the method for omeprazole in plasma more than 25,000 analyses have been performed during the last five years. This large number of analyses also initiated the development of a totally automatic method based on Technicon's FAST®-LC system [7].

Recently, a paper on the reversed-phase LC determination of omeprazole has been published [8].

EXPERIMENTAL

Apparatus

The liquid chromatograph was composed of an Altex 110A LC-pump (Altex Scientific, Berkeley, CA, U.S.A.) and an LDC Spectromonitor III (Laboratory Data Control, Riviera Beach, FL, U.S.A.) UV detector. The automatic injector was either a Waters WISP 710B (Waters Assoc., Milford, MA, U.S.A.) or a Kontron MSI 660 (Kontron Electrolab, London, U.K.) using an injection volume of 150 μl. The separation columns were of precision-bore stainless steel (150 × 4.5 mm) with end fittings of modified Swagelok connections and were home-packed either with LiChrosorb Si 60, 5 μm (E. Merck, Darmstadt, F.R.G.) or with Polygosil C_{18}, 5 μm (Macherey-Nagel, Düren, F.R.G.). In the reversed-phase system a precolumn was used (Brownlee Labs, Spheri-5, RP-8, 30 × 4.6 mm).

Reagents

Methylene chloride, methanol and ammonium hydroxide solution (25%) (pro analysi grade, Merck) were used. Acetonitrile was of HPLC grade (Rathburn Chemicals, Scotland, U.K.). All reagent and buffer solutions were prepared with analytical reagent grade chemicals. Omeprazole (Hässle, Mölndal, Sweden) fulfilled the quality requirements of the Pharmacopoeia Nordica.

Omeprazole, the metabolites and internal standards were supplied by the Department of Organic Chemistry, Hässle. For chemical structures, see Fig. 1.

Standard solutions

A standard solution for plasma determination of omeprazole and metabolites (60 μmol/l) was prepared by dissolving 2 mg of each compound in 20 ml of methanol and diluting to 100 ml with carbonate buffer pH 9.3, I = 0.1. A 100-μl volume of the standard solution was added to a large number of 5-ml centrifuge tubes which were kept frozen at −18°C for no longer than three months. Plasma standards were prepared by adding 1 ml of blank plasma to the tubes containing standard solution, at the time of analysis.

For the urine analysis, the standard solution used had double the concentration of each compound (120 μmol/l). Again, 100 μl of standard solution were stored at −18°C in centrifuge tubes and 1 ml of urine was added to make a urine standard, at the time of analysis.

Stock solutions of internal standard for omeprazole in plasma (VI) and for hydroxyomeprazole in plasma and urine (V) contained 5—10 mg per 100 ml of methanol—carbonate buffer, and were kept in a refrigerator for not more than one month.

Sample preparation

Plasma. The plasma method was optimized for the determination of omeprazole and the sulphone. The concentration of the sulphide in human plasma is usually too low to be determined. The frozen plasma sample is allowed to thaw at room temperature and is mixed and centrifuged. A 1-ml aliquot of the sample is transferred to a centrifuge tube, mixed with 100 μl of sodium dihydrogen phosphate 1 mol/l (final pH 6.5—7.0) and 100 μl of the internal standard solution (VI) and is then extracted with 1 ml of methylene chloride by shaking for 10 min. After centrifugation twice for 10 min at 2500 *g*, the aqueous upper layer is aspirated and discarded. Part of the organic layer is transferred to sample vials for the automatic injector; 150 μl are injected onto the normal-phase LC column (Fig. 2).

In certain series of plasma samples it was also of interest to determine the content of the sulphide (III); for example, if the sulphide had been given as a drug, in which case V was used as internal standard instead of VI. Furthermore, a slight modification in mobile phase composition was made (Fig. 3).

For the determination of the more hydrophilic hydroxy metabolite (IV) in plasma, the following procedure was used. The frozen plasma sample is allowed to thaw at room temperature and is mixed and centrifuged. Then 1 ml of the sample is transferred to a centrifuge tube, mixed with 100 μl of sodium dihydrogen phosphate 1 mol/l (final pH 6.5—7.0) and 100 μl of the internal standard (V), and is then extracted into 10 ml of methylene chloride by shaking for 10 min. After centrifugation for 10 min (2500 *g*), the aqueous layer is aspirated and discarded. An 8-ml volume of the organic layer is transferred to a conical centrifuge tube and evaporated under nitrogen flow. The residue is dissolved in 500 μl of 20% acetonitrile + 80% phosphate buffer pH 7.5, I = 0.05, and 150 μl are injected by means of the automatic injector onto the reversed-phase column (Fig. 4).

Urine (omeprazole, its sulphone and hydroxyomeprazole). The procedure is the same as for the determination of hydroxyomeprazole in plasma (Fig. 5).

Chromatography

The chromatographic separation for the plasma method (omeprazole and the sulphone) is made on a silica column with a mobile phase of methylene chloride containing 3.5% of a solution of 5% of concentrated ammonium hydroxide in methanol. For the determination of omeprazole sulphide (III) the methanol content was decreased to 2.0%. The flow-rate was 1.5 ml/min and the eluent was monitored by UV detection at 302 nm.

In the method for hydroxyomeprazole in plasma and for omeprazole and metabolites in urine, a reversed-phase system is used with a mobile phase containing acetonitrile and phosphate buffer pH 7.5 (I = 0.05) (30:70, v/v). The flow-rate was 1 ml/min and the detector wavelength the same as in the normal-phase method.

Quantification is based on peak height measurements and internal standardization.

Determination of distribution ratios

The distribution ratios for omeprazole, the sulphone (II), the sulphide (III), the hydroxy metabolite (IV) and the internal standards (V and VI) between methylene chloride and water at pH 6.5—7.0 were determined by equilibration in centrifuge tubes. As aqueous phase, phosphate buffer solutions (I = 0.10) were used. After phase separation by centrifugation, the concentration of the compounds in the organic phase was determined by LC. In the aqueous phase the concentration was determined after repeated extraction of an aliquot by methylene cloride, and measurement in the organic phase.

Stability

Standard solutions of omeprazole were kept at pH 9 to ensure good stability during storage. The stability of omeprazole at −18°C in plasma at pH 7.5 and pH 9 was studied. Authentic plasma samples were divided in two parts, and carbonate buffer was added to one of the samples to give a final pH 9. The two samples were then divided into several samples to provide a sufficient number of samples for a long-term study. Samples were then analysed over a period of one year.

Another study was performed in which authentic plasma samples stored at room temperature for 0, 1, 2, 3 and 4 days were analysed for omeprazole.

RESULTS AND DISCUSSION

Extraction

Omeprazole, the metabolites discussed here and the internal standards V and VI are ampholytes and have two dissociation constants: 2—5 for the pyridine nitrogen and 8—11 for the imidazole nitrogen (Table I). This means that a pH between 6 and 7 is appropriate for extraction of the compounds into an organic phase. Omeprazole is easily extracted into methylene chloride. The distribution ratios for the compounds studied are shown in Table I. Using equal

TABLE I

DISTRIBUTION RATIOS (D) OF OMEPRAZOLE, METABOLITES AND INTERNAL STANDARDS BETWEEN METHYLENE CHLORIDE AND PHOSPHATE BUFFER SOLUTIONS

pH 6.5—7.0, $I = 0.10$. pK^* is the mixed dissociation constant.

Compound	D	$pK^{*\,*}_{(1)}$	$pK^{*\,**}_{(2)}$
Omeprazole (I)	180	4.2	9.0
Sulphone (II)	300	3.5	7.8
Sulphide (III)	3100	5.2	11.5
Hydroxyomeprazole (IV)	2.4	2.1	9.0
V	450	4.2	9.2
VI	160	4.3	9.4

*$pK^*_{(1)}$ refers to the pyridine nitrogen [9].

**$pK^*_{(2)}$ refers to the benzimidazole nitrogen [9].

phase volumes, the theoretical absolute recovery is ⩾ 99% for all of the compounds except for the hydroxy metabolite. For the extraction from plasma, equal phase volumes were used, while an eight times larger volume of organic phase was needed to obtain a high recovery for the more polar hydroxy metabolite.

Chromatography

Normal-phase systems. In the determination of omeprazole in plasma a normal-phase separation system was chosen since the sample work-up could be limited to extraction into methylene chloride and injection of an aliquot of the extract onto the LC column. Moreover, the chromatograms are relatively free from interfering peaks. The mobile phase contains methylene chloride as main component as in the extraction solvent, and with dilute ammonia solution in methanol as modifier. The concentration of ammonia is sufficiently low not to be deleterious for the stationary phase and the columns show good long-term stability. The normal-phase method is optimized for the determination of omeprazole and its sulphone (II), the latter being subject to slight interference by an adjacent peak (Fig. 2). In a limited number of studies the sulphide had to be determined and the methanol content was then lowered from 3.5% to 2.0% to give this compound a suitable retention. Using this phase, omeprazole and the sulphone could be determined as well, but with a 2—3 times lower sensitivity because of the larger retention volume (Fig. 3).

Reversed-phase methods. In urine, the compound of most interest seems to be the hydroxy metabolite (IV) [6], and the analytical method was thus focused on the determination of this compound. A reversed-phase system was chosen in which the hydroxy metabolite (IV) elutes ahead of omeprazole and the sulphone. The pH of the mobile phase was 7.5 to ensure stability of both the compounds and the stationary phase. After extraction into methylene chloride and evaporation of the solvent, the residue is dissolved in a solvent containing a lower content of acetonitrile than in the mobile phase in order to obtain a concentration in the starting zone of the column. For the determination of hydroxyomeprazole in plasma the same sample preparation and chromatographic system were used (Fig. 4).

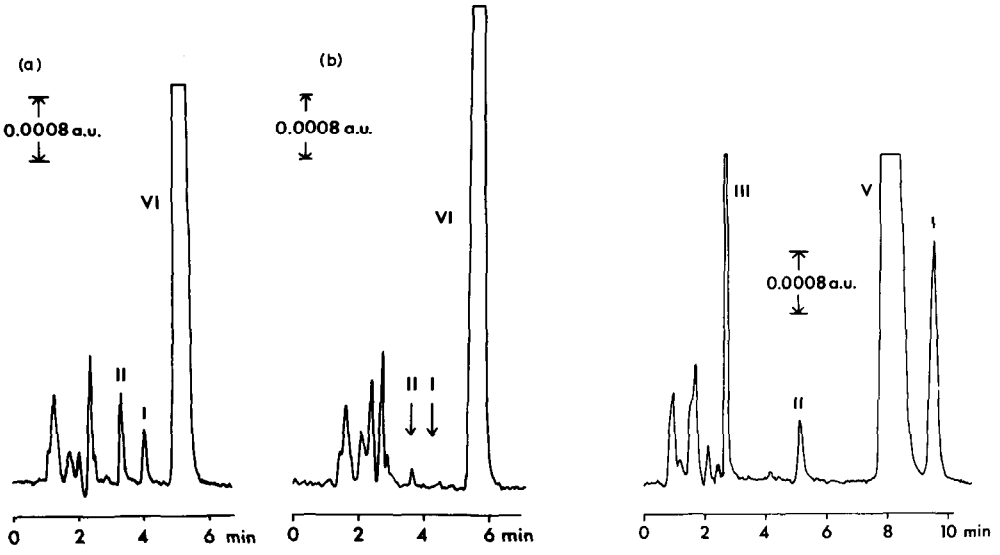

Fig. 2. Omeprazole (I) and the sulphone (II) in a plasma sample from a patient administered omeprazole. Packing material: LiChrosorb Si 60, 5 μm. Mobile phase: methanol (containing 5% of 25% ammonium hydroxide)—methylene chloride (3.5:96.5, v/v). (a) Sample: 150 μl of an extract from 1 ml of plasma containing omeprazole (I) 70 nmol/l and the sulphone (II) 110 nmol/l. (b) Sample: blank plasma.

Fig. 3. Omeprazole (I), the sulphone (II) and the sulphide (III) in a plasma sample from a patient administered omeprazole. Packing material: LiChrosorb Si 60, 5 μm. Mobile phase: methanol (containing 5% of 25% ammonium hydroxide)—methylene chloride (2.0:98.0, v/v). Sample: 150 μl of an extract from 1 ml of plasma containing the sulphide (III) 910 nmol/l, the sulphone (II) 170 nmol/l and omeprazole (I) 1300 nmol/l.

Selectivity

In both the normal-phase and the reversed-phase systems the separation between omeprazole and its main metabolites — the sulphone, sulphide and hydroxy compound — and the internal standards used was quite sufficient (Figs. 2—5). There is no interference in the chromatograms from the H_2-receptor antagonists, cimetidine and ranitidine, if they by chance should be present in the sample.

Stability

The results of the stability studies of omeprazole and its sulphone in authentic plasma samples (Table II) show clearly that plasma samples of omeprazole can be stored without any significant degradation at −18°C for one year. No significant difference was seen between storage at pH 9 and pH 7.5. Neither did four days at room temperature produce any degradation.

Urine samples for analysis were collected in bottles containing 2.5 ml of 1 mol/l sodium carbonate per hour of collection period, to buffer the urine to at least pH 8.

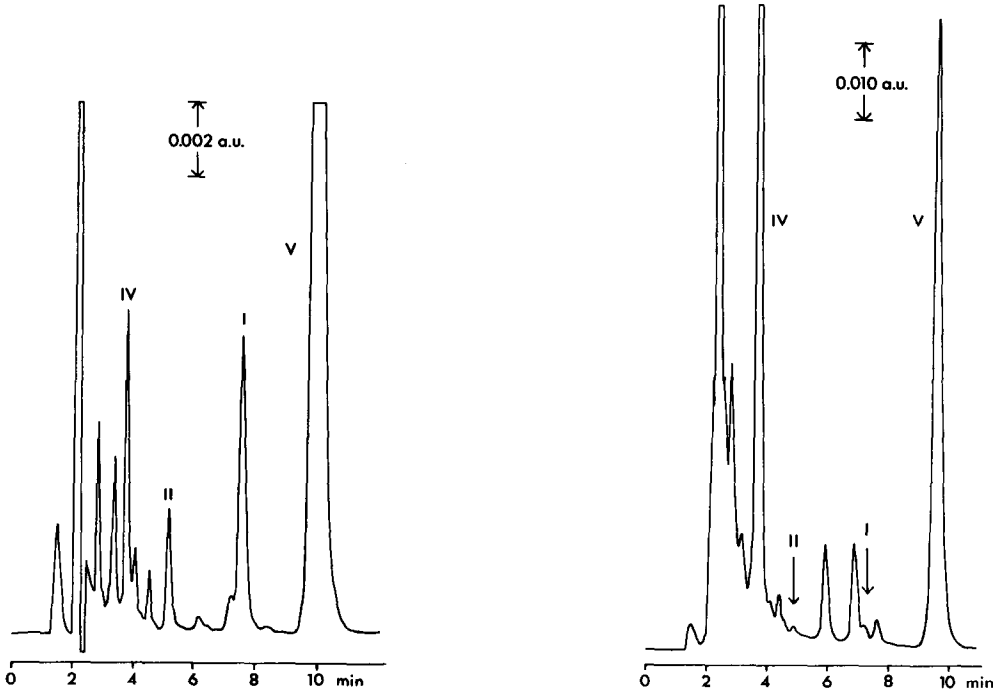

Fig. 4. Omeprazole (I), the sulphone (II) and the hydroxy metabolite (IV) in a plasma sample from a patient administered omeprazole. Packing material: Polygosil C_{18}, 5 μm. Mobile phase: 30% of acetonitrile in phosphate buffer pH 7.5, I = 0.05. Sample: 150 μl of an extract from 1.0 ml of plasma containing omeprazole (I) 650 nmol/l, the sulphone (II) 170 nmol/l and hydroxyomeprazole (IV) 350 nmol/l.

Fig. 5. Omeprazole (I), the sulphone (II) and the hydroxy metabolite (IV) in a urine sample from a patient administered omeprazole. Packing material: Polygosil C_{18}, 5 μm. Mobile phase: 30% of acetonitrile in phosphate buffer pH 7.5, I = 0.05. Sample: 150 μl of an extract from 1.0 ml of urine containing hydroxyomeprazole (IV) 5.5 μmol/l and < 100 nmol/l of omeprazole (I) and the sulphone (II).

Recovery and repeatability

Ten identical plasma samples containing either omeprazole, the sulphone and VI or the sulphide and V were analysed and the results were compared to direct injections of the corresponding compounds dissolved in methylene chloride. The results for the absolute recovery and the repeatability are given in Table III. As can be seen, the recovery for all of the compounds is more than 95%. The repeatability for omeprazole, the sulphone and sulphide is excellent. The minimum determinable concentration, defined as the level at which the relative standard deviation is 10—15%, is about 20 nmol/l for omeprazole and the sulphone, and about 50 nmol/l for the sulphide.

The recovery and repeatability for hydroxyomeprazole in plasma after reversed-phase chromatography are also shown in Table III.

The linearity ranges from 25—50 nmol/l to 50—100 μmol/l of plasma or urine.

In urine, the recovery and repeatability for omeprazole, the sulphone, the

TABLE II

STABILITY OF OMEPRAZOLE (I) AND THE SULPHONE (II) IN TWO PLASMA SAMPLES FROM PATIENTS ADMINISTERED OMEPRAZOLE

A: stored at −18°C at pH 7.5 and 9. B: stored in the dark at room temperature (+23°C). Initial concentration of omeprazole 2 μmol/l and of the sulphone 0.5 μmol/l.

Storage time		pH	Percentage of initial concentration	
			Omeprazole	Sulphone
A	1 (weeks)	7.5	100	100
		9	100	100
	5	7.5	104	97
		9	107	101
	15	7.5	102	101
		9	103	104
	52	7.5	102	99
		9	104	103
B	0 (days)	7.5	100	100
	1	7.5	103	97
	2	7.5	109	114
	3	7.5	110	104
	4	7.5	101	109

TABLE III

REPEATABILITY AND RECOVERY IN THE DETERMINATION OF OMEPRAZOLE, METABOLITES AND INTERNAL STANDARDS IN PLASMA AND URINE

The recovery given is the absolute recovery (n = 10).

Compound	Concentration (μmol/l)	Repeatability (S.D., %)	Recovery (%)	
			Calculated	Obtained
Plasma				
Omeprazole (I)	3.00	1.1	99	100
	0.30	3.5	99	98
	0.03	9.7	99	95
Sulphone (II)	2.60	1.5	99	98
	0.10	5.5	99	94
Sulphide (III)	6.30	1.0	99	102
	0.16	2.6	99	96
Hydroxyomeprazole (IV)	3.00	1.5	92	90
	0.10	5.7	92	87
V	3.00	1.5	99	98
VI	3.00	1.5	99	95
Urine				
Omeprazole (I)	100	1.5	99	93
	10	3.2	99	95
Sulphone (II)	10	1.5	99	95
Hydroxyomeprazole (IV)	10	1.5	92	90
	0.2	4.8	92	87
V	10	1.2	99	95

hydroxy metabolite and the internal standard V were determined in the same manner as in plasma. The results are shown in Table III. All recoveries are better than 95%, except for hydroxyomeprazole which has a recovery of only 85%. The actual concentrations of omeprazole and the sulphone in authentic samples are very low. The amounts excreted are much less than 1% of the given dose. The concentration of the main metabolite in urine, hydroxyomeprazole, is more than ten times higher. The minimum determinable concentration is 30 nmol/l for the hydroxy compound and 50 nmol/l for omeprazole and the sulphone, using 1 ml of urine.

Method reproducibility

The long-term reproducibility of the main plasma method was studied by analysis of identical samples. A large number of drug-free plasma samples was spiked with the same concentration of omeprazole and kept frozen for up to five months. During this time two samples were analysed each day of analysis. The results from five studies are given in Table IV and show that the mean value of each study lies between 99% and 103% of the nominal value, with a relative standard deviation of 3—4.5%.

TABLE IV

REPRODUCIBILITY OF THE NORMAL-PHASE PLASMA METHOD FOR OMEPRAZOLE

Identical plasma samples (spiked samples) were analysed, two samples per day of analysis, over a longer period of time. $\overline{m}$ = mean value of the results from each study expressed as a percentage of the nominal value. Concentration of omeprazole = 5 μmol/l.

Study No.	n	$\overline{m}$ (%)	S.D. (%)
A 106	94	99	4.0
A 109	92	104	3.3
A 203	42	101	4.5
A 207	64	99	4.3
A 211	67	99	3.5

Method comparison

Comparisons were made between two separate LC methods for omeprazole assay in plasma, the normal-phase method and a method based on Technicon's

TABLE V

COMPARISON BETWEEN A FAST-LC METHOD [7] AND THE NORMAL-PHASE METHOD FOR DETERMINATION OF OMEPRAZOLE IN PLASMA

Plasma samples from patients administered omeprazole were analysed.

Study No.	Mean ratio (FAST-LC/ normal phase)	S.D. (%)	n
1	0.97	7.0	61
2	1.01	8.7	100
3	0.95	7.0	45

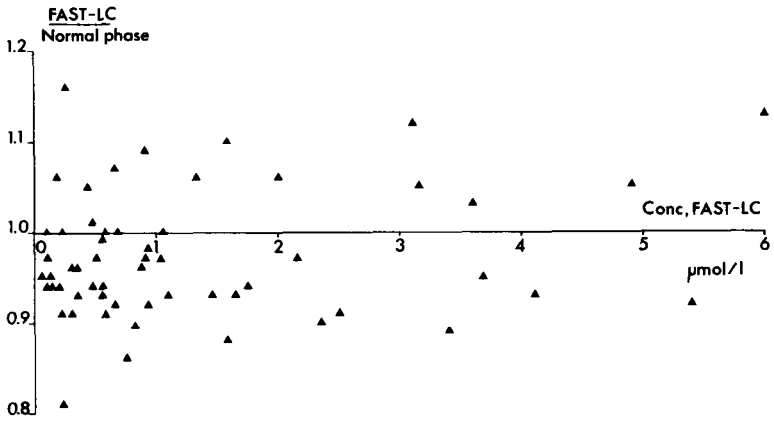

Fig. 6. Comparison between a FAST-LC method [7] and the normal-phase method for the determination of omeprazole in plasma. Plasma samples are from patients administered omeprazole. n = 61, $\overline{m}$ = 0.97, S.D. = 7.0%.

Fully Automated Sample Treatment LC, FAST-LC [7]. The main differences between the methods are that the FAST-LC method comprises a fully automated sample work-up step including extraction in coils into isopropanol—chloroform, evaporation, dissolution in a dilute mobile aqueous phase and injection onto a reversed-phase LC column. The results from three studies are presented in Table V. As can be seen, the mean quotients of the results of the two methods are around 1.00 and the standard deviation of the quotients 7—9%. The concentration of the samples varied from 0.1 to 10 μmol/l of plasma. One comparative study is featured in Fig. 6. A detailed presentation of the FAST-LC method will be published shortly [7].

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