Journal of Chromatography, 339 (1985) 139—147
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2481

DETERMINATION OF CIMETIDINE AND METABOLITES IN PLASMA BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC RADIAL COMPRESSION TECHNIQUE*

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(First received August 15th, 1984; revised manuscript received November 22nd, 1984)

SUMMARY

A high-performance liquid chromatographic method for the detection of cimetidine and its metabolites in plasma was developed which has a short analysis time and good resolution. The total analysis time was approximately 11 min. The standard curves were linear over the concentrations used for all compounds and the sensitivity limits were good. The coefficient of variation for within-day and between-day analysis was less than 4.2% for all compounds with the exception of guanyl urea cimetidine, which was approximately 10%. Currently, this assay is being used in a pharmacokinetic study of plasma and gastric aspirate samples obtained from a critically ill pediatric population.

INTRODUCTION

Since the original description by Randolph et al. [1] of a high-performance liquid chromatographic (HPLC) method for the detection of cimetidine in

*Presented at the 35th National Meeting of the American Pharmaceutical Association, Academy of Pharmaceutical Sciences, Miami Beach, FL, U.S.A., November 13—17, 1983.

0378-4347/85/$03.30

blood and urine, improvements have been made in the areas of sample size, mobile phase and columns [2—11]. Randolph et al. [1] described an HPLC method using a 5-μm silica steel column and a mobile phase consisting of acetonitrile—methanol—water—ammonium hydroxide (1000:60:20:5). Until recently, HPLC methods have focused on the detection of cimetidine only. Ziemniak et al. [11] described a procedure which allowed for the detection of cimetidine and its three major metabolites: cimetidine sulfoxide (CSOX), hydroxymethyl cimetidine (OHMC), and guanyl urea cimetidine (GUC). The major disadvantages of this procedure are its complex extraction and lengthy analysis time (i.e., 26 min). The mobile phase used by Ziemniak et al. [11] as well as the ones used by Randolph et al. [1] and by Chairmonte and Schentag [2] have a final pH of 10 which has a deleterious effect on instrumentation and potentially reduces column life. This paper describes a HPLC assay utilizing radial compression to achieve higher flow-rates resulting in a shorter analysis time for the detection of cimetidine and its metabolites.

EXPERIMENTAL

Instrumentation

A constant-flow solvent delivery system (Waters M-45, Waters Assoc., Milford, MA, U.S.A.) was used in combination with a radial compression unit (Waters Z-module, Waters Assoc.) and an ultraviolet fixed-wavelength detector (Beckman Model 160) at 229 nm. Results were recorded on a Linear Model 591 strip chart recorder (Linear Instruments, Reno, NV, U.S.A.). Separation was performed using a μBondapak C_{18}, 10-μm, radial compression cartridge (Waters C_{18}, Waters Assoc.).

Chemicals and reagents

Cimetidine, N''-cyano-N-methyl-N'-[2-[[(5-methyl-1H-imidazol-4-yl)methyl]-thio]-ethyl]-guanidine (Tagamet), the metabolites (CSOX, OHMC and GUC) and the internal standard (I.S.), N:methyl-N'-[(3-imidazol-4-yl)propyl]-cyanoguanidine, were donated by Smith Kline and French Labs. (Philadelphia, PA, U.S.A.). The mobile phase consisted of water—methanol (80:20) and was prepared by mixing glass-double-distilled water with 99% *n*-butylamine (5 m*M*) (Aldrich, Milwaukee, WI, U.S.A.) and adjusting the pH of the solution to 7.1 with phosphoric acid. Certified HPLC-grade methanol (MCB Reagents, EM Industries, Gibbstown, NJ, U.S.A.) was then added and the final mixture was filtered and degassed.

Chromatography

Cartridges were conditioned by wetting the column with acetonitrile for 30 min at a flow-rate of 4.0 ml/min. The solvent was then switched to methanol—water (50:50) for an additional 30 min followed by mobile phase at 4.0 ml/min for 1 h before analysis was begun. All assays were done at 0.005 absorbance units full scale and a chart speed of 20 cm/h. Since the assay procedure was being utilized for the detection of cimetidine and metabolites in patient plasma samples, there were periods of time when the system was not utilized. Due to the addition of *n*-butylamine to the mobile phase, the

column was flushed with water followed by a solution of methanol—water (50:50) adjusted to a pH of 4 with acetic acid to retain resolution. Pressures of 70—76 bars were maintained throughout the analysis and pressures above 90 bars required cleaning of in-line filters.

Standard curves

Stock solutions were prepared in distilled water at the following concentrations: cimetidine 40 μg/ml, CSOX 40 μg/ml, OHMC 60 μg/ml, GUC 90 μg/ml, I.S. 30 μg/ml. Working standard solutions were prepared by diluting the stock solutions with water (1:1 and 1:10). Plasma standards were prepared by spiking known quantities of cimetidine and the metabolites in 0.5 ml of pooled human plasma containing 600 ng of the internal standard. Five calibration samples were used at the following concentration range: cimetidine 0.2—4.0 μg/ml, CSOX 0.08—1.2 μg/ml, OHMC 0.3—4.5 μg/ml, GUC 0.45—9 μg/ml. The plasma standard curve was constructed by plotting the peak height ratios versus the weight content of cimetidine or the metabolites.

Analytical recovery

The extraction procedure used in this assay has been previously described by Ziemniak et al. [11]. Extraction recovery was performed to determine the absolute percentage recovery in our laboratory using three known concentrations.

The parent compound and its metabolites were added to plasma and a solution of mobile phase containing the internal standard. The plasma samples were then extracted, the internal standard was added and the mixture vortexed just prior to injection. Five replicate determinations were performed at each concentration and the percentage recovery determined by comparing the peak height ratios for the extracted samples to the values obtained from the unextracted mobile phase standards. All analytical and patient plasma samples were kept at −55°C until assayed.

Sensitivity study

The sensitivity of the assay was determined by injecting five known concentrations into the system. The sensitivity limit for each compound was defined as a peak at least four times the height of the baseline noise of the recorder. Each sample was reconstituted with 100 μl of mobile phase and the entire volume was injected after thoroughly vortexing the sample.

Precision analysis

Three concentrations were selected for determination of assay variability. Four extracted samples of each concentration were assayed five times throughout a single day to determine the within-day variation. Four samples of each concentration were extracted daily and assayed in duplicate for five consecutive days to determine between-day variations.

Drug-assay interference

Currently, this assay is being utilized to determine concentrations of cimetidine and its metabolites in a critically ill pediatric population. Therefore,

TABLE I

PRESELECTED PLASMA CONCENTRATIONS FOR DRUG-ASSAY INTERFERENCE

Commercial products were used.

Drug	Low concentration (μg/ml)	High concentration (μg/ml)
Gentamicin	4	8
Tobramycin	4	8
Chloramphenicol	5	20
Penicillin-G	20	80
Ampicillin	50	100
Ticarcillin	100	200
Nafcillin	4	16
Oxacillin	8	32
Phenytoin	10	20
Phenobarbital	20	40

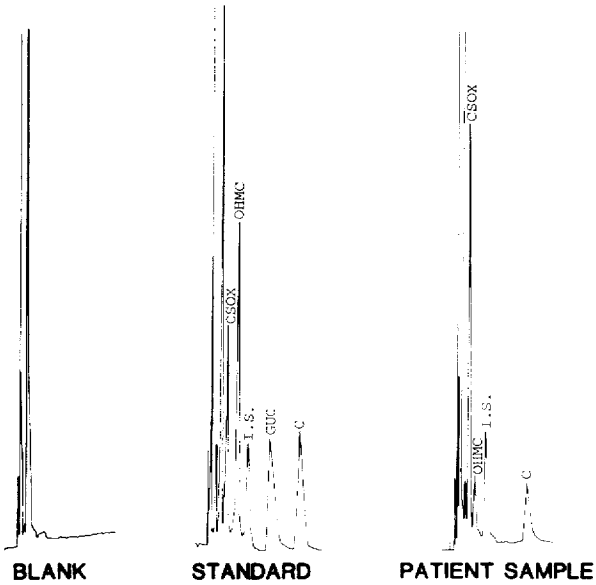
baseline blood was obtained, when possible, from patients prior to the initiation of cimetidine therapy for the detection of chromatographic interference caused by concomitant drugs or their metabolites. In addition, a set of drugs which are commonly administered in conjunction with cimetidine in this patient population were identified and injected onto the column separately to determine if interference would occur (Table I). Each of the drugs were added to pooled human plasma to achieve the preselected concentrations. An aliquot of 0.5 ml was then extracted and later reconstituted with 100 μl of mobile phase. The lowest concentration of each drug was injected onto the column in a volume of 10 and 25 μl. If no peaks were observed at the lower concentrations, the higher concentrations were injected using the same volumes.

RESULTS

The standard curves for all compounds studied were linear over the plasma concentrations selected as determined by linear regression analysis. All data interpretation from the curves was determined by mathematical calculations. Fig. 1 shows examples of a pooled human plasma bank, a plasma standard and a pediatric patient (T = 2 h, cimetidine 5.0 mg/kg intravenous infusion over 15 min). Listed below the chromatographs are the compounds and their corresponding retention times and sensitivity limits.

The results of the analytical recovery of cimetidine and its metabolites at three concentrations appear in Table II. The extraction efficiency was determined to be 70—87% for cimetidine and ranged between 65% to 89% for its metabolites.

Reproducibility studies revealed within-day variations of less than 2.8% for all compounds except for GUC in which the coefficient of variation was 8.5% at the lower concentration and 7.6% at the high concentration (Table III). The between-day variation for five consecutive days was determined to be less than 4.2% for cimetidine, CSOX, and OHMC. For GUC the coefficient of variation was approximately 10% for all concentrations (Table IV).



Compound	Sensitivity (μg/ml)	Retention time (min)
Cimetidine sulfoxide (CSOX)	0.04	2.4
Hydroxymethyl cimetidine (OHMC)	0.06	3.3
Internal standard (I.S.)	-	5.1
Guanyl urea cimetidine (GUC)	0.18	6.9
Cimetidine (C)	0.04	10.8

Fig. 1. Sample chromatogram, retention times and sensitivity limits.

TABLE II

ANALYTICAL RECOVERY OF CIMETIDINE AND ITS METABOLITES

Compound	Extraction recovery		
	Concentration (μg/ml)	Percentage recovery (Mean ± S.D.)	C.V. (%)
Cimetidine	0.22	87.3 ± 4.6	5.3
	1.65	70.1 ± 2.4	3.4
	9.71	71.7 ± 1.6	2.2
Cimetidine sulfoxide	0.10	70.5 ± 1.9	2.7
	2.32	84.3 ± 1.4	1.7
	10.40	89.4 ± 3.0	3.4
Hydroxymethyl cimetidine	0.19	79.0 ± 1.2	1.5
	1.35	69.3 ± 1.7	2.5
	4.69	73.5 ± 1.5	2.0
Guanyl urea cimetidine	0.28	64.8 ± 3.4	5.3
	1.62	66.9 ± 1.9	2.8
	8.77	70.1 ± 3.3	4.8

TABLE III

WITHIN-RUN PRECISION OF CIMETIDINE AND METABOLITES

$n = 5$.

Compound	Concentration (μg/ml, mean ± S.D.)	C.V. (%)
*Subtherapeutic**		
Cimetidine	0.20 ± 0.004	2.1
Cimetidine sulfoxide	0.08 ± 0.001	1.8
Hydroxymethyl cimetidine	0.33 ± 0.009	2.8
Guanyl urea cimetidine	0.45 ± 0.038	8.5
*Therapeutic**		
Cimetidine	1.15 ± 0.020	1.7
Cimetidine sulfoxide	0.46 ± 0.006	1.4
Hydroxymethyl cimetidine	1.62 ± 0.039	2.4
Guanyl urea cimetidine	2.28 ± 0.176	7.7
*Supratherapeutic**		
Cimetidine	3.75 ± 0.068	1.8
Cimetidine sulfoxide	1.11 ± 0.014	1.3
Hydroxymethyl cimetidine	4.70 ± 0.122	2.6
Guanyl urea cimetidine	9.45 ± 0.718	7.6

*Based on plasma cimetidine concentration.

TABLE IV

BETWEEN-RUN PRECISION OF CIMETIDINE AND METABOLITES

$n = 5$.

Compound	Concentration (μg/ml, mean ± S.D.)	C.V. (%)
*Subtherapeutic**		
Cimetidine	0.21 ± 0.009	4.2
Cimetidine sulfoxide	0.078 ± 0.002	2.6
Hydroxymethyl cimetidine	0.32 ± 0.012	3.9
Guanyl urea cimetidine	0.48 ± 0.049	10.2
*Therapeutic**		
Cimetidine	1.12 ± 0.041	3.7
Cimetidine sulfoxide	0.46 ± 0.010	2.1
Hydroxymethyl cimetidine	1.58 ± 0.055	3.5
Guanyl urea cimetidine	2.16 ± 0.203	9.4
*Supratherapeutic**		
Cimetidine	3.87 ± 0.128	3.3
Cimetidine sulfoxide	1.10 ± 0.023	2.1
Hydroxymethyl cimetidine	4.63 ± 0.167	3.6
Guanyl urea cimetidine	8.75 ± 0.858	9.8

*Based on plasma cimetidine concentration.

Plasma samples were obtained from several patients prior to receiving cimetidine and no chromatographic interference with the compounds of interest was noted. These patients were receiving the following drugs: phenytoin,

phenobarbital, dexamethasone, ampicillin, penicillin G, and morphine sulfate. Of the ten drugs evaluated in vitro for potential assay interference, only two penicillins were noted to elute in the range of the normal analysis. Penicillin G eluted at 5.4 min and ampicillin at 12.1 min. Based on a standard curve run concomitantly, the peaks for CSOX, OHMC, I.S., GUC and cimetidine were 2.4, 3.6, 4.2, 5.8 and 10.9 min, respectively.

DISCUSSION

This assay alleviates two problems associated with the detection of cimetidine and its metabolites by other methods, namely lengthy analysis time and an adverse mobile phase. Ziemniak et al. [11] were the first to describe a method capable of detecting cimetidine and its metabolites, but at the cost of an analysis time of 25.8 min. A few assays use a mobile phase consisting of a combination of acetonitrile, methanol, water and ammonium hydroxide with an apparent pH of 10.5. Such alkaline solutions have a deleterious effect on columns and instrumentation. The method described here uses a mobile phase comprised of methanol and water with an apparent pH of 7.1. This assay utilized radial compression which allowed for a higher flow-rate resulting in a shorter analysis time. The sensitivity limit of cimetidine is similar to previously reported values of 0.05—0.1 μg/ml.

The percentage recovery of the drug and metabolites achieved by Ziemniak et al. [11] were higher than those observed in our laboratory and may be accounted for by their inclusion of pipette transfer losses in the total recovery determination [12]. Both within-day and between-day reproducibility was good for all compounds with the exception of GUC. The large coefficient of variation observed with GUC would prohibit the use of this assay for the determination of this metabolite's concentration in plasma.

Although previous investigators [7—10] utilizing simplified extraction procedures have demonstrated accuracy and reproducibility for the detection of cimetidine, interfering substances would preclude the detection and resolution of cimetidine metabolites. The simplified extraction procedures consisted of either an organic liquid or solid phase separation in a minicolumn, evaporation under a stream of nitrogen, followed by reconstitution and analysis. Total time of analysis by these procedures is approximately 30—40 min.

The drug-assay interference component revealed two penicillins which eluted in the normal range of the analysis. Penicillin G appeared near the GUC peak and therefore the potential for interference exists; however, GUC was never observed in patient samples. Although ampicillin eluted near the cimetidine peak, resolution between the two compounds was sufficient for differentiation. Patient plasma samples obtained prior to the institution of cimetidine therapy assisted in the evaluation of interfering peaks secondary to metabolites of concomitantly administered medications.

An example of the steady-state plasma elimination curves for cimetidine, CSOX and OHMC in a child admitted to the clinical study because of a closed head injury, secondary to a motor vehicle accident, appears in Fig. 2. The disappearance of the parent compound is characterized by an initial rapid distribution phase followed by a slower terminal elimination. The half-lives

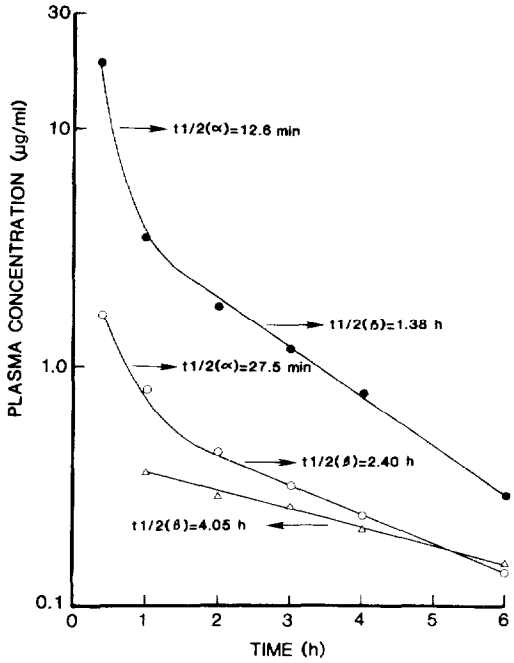


Fig. 2. Plasma elimination curves for cimetidine (•), cimetidine sulfoxide (○), and hydroxymethyl cimetidine (△) in a seven-year-old child given 8 mg/kg cimetidine administered intravenously as a 20-min infusion.

for the metabolites, CSOX and OHMC, were prolonged in comparison to the parent compound. The GU metabolite has not been observed in any of the 29 patients admitted to the clinical study. The suggested therapeutic range for cimetidine is 0.5—1.0 μg/ml.

Radial compression HPLC is an acceptable technique for the rapid and reliable determination of cimetidine and its metabolites in plasma. Currently, this assay is being used for the determination of the above compounds in a critically ill population.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of Dr. Howie James, Senior Technical Consultant, Waters Assoc. and Ms. Kimberly Zainfeld for her secretarial assistance. This work was supported in part by Biomedical Research Support Grant (No. RR-05855), National Institutes of Health, and a grant from Smith Kline and French Labs., Philadelphia, PA, U.S.A.

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