Journal of Chromatography, 496 (1989) 472–477
Biomedical Applications
Elsevier Science Publishers B V , Amsterdam — Printed in The Netherlands

CHROMBIO 4968

Note

High-performance liquid chromatographic method with fluorescence detection for the simultaneous determination of metopimazine and its acid metabolite in serum

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(First received April 4th 1989, revised manuscript received July 24th, 1989)

Emesis and vomiting is a great problem for many patients undergoing treatment with cytostatic agents. The phenothiazine derivative metopimazine (MPZ), in doses below the toxic level, has demonstrated a certain antiemetic effect compared with a placebo in treatment of these patients [1].

In order to determine the relevant dose level and dose interval, and thereby perhaps improve the therapeutic effect, we needed a relatively simple method for measuring MPZ and its acid metabolite (AMPZ). In a previously high-performance liquid chromatographic (HPLC) method (Analytical Method Report IBP/Biodyn. No. 527, unpublished), MPZ and AMPZ were determined separately. This paper describes an HPLC method with a relatively simple extraction procedure. Using a cyano column and fluorescence detection, MPZ and AMPZ could be determined simultaneously with sufficient sensitivity, precision and selectivity.

0378-4347/89/$03 50

EXPERIMENTAL

Chemicals

MPZ, AMPZ and their internal standards (I.S.$_{MPZ}$, 17 979 RP and I S.$_{AMPZ}$ 43 080 RP) were gifts from Rhône-Poulenc Santé (Antony, France). Molecular structures are shown in Fig. 1. Methanol was of HPLC grade (LiChrosolv from E. Merck, Darmstadt, F.R.G.). All other solvents and chemicals were of analytical quality.

HPLC conditions

The chromatograph consisted of a Waters Model 6000A pump, a Waters WISP 710B automatic injection system (Waters Assoc, Milford, MA, U.S.A.) and a Model F 1000 Hitachi fluorescence detector, operating at 280 nm (excitation and 500 nm (emission) (Hitachi E Merck). The analytical column was a 5-μm Supelcosil CN 25 cm $\times$ 4.6 mm I.D. (Supelco, Gland, Switzerland) combined with a LiChroCART 4 $\times$ 4 LiChrosorb CN guard column (Merck), used at room temperature.

The mobile phase was methanol–0.1 *M* ammonium acetate (50 50) The flow-rate was 1.5 ml/min.

Procedure

Blood samples were collected in glass tubes by venipuncture. The serum was separated and stored at $-20\,^{\circ}C$ until the time of analysis.

To 1.0 ml of serum were added 1.0 ml of 50 m*M* perchlorate buffer (pH 2.5), 50 μl of internal standard solution (4.8 μg/ml I.S.$_{MPZ}$ and 2.4 μg/ml I S $_{AMPZ}$) and 9.0 ml of chloroform. The sample was shaken for 15 min, then most of the aqueous phase aspirated off. After centrifugation at 1300 *g* for 5 min the remaining aqueous phase was aspirated off and discarded. The chloroform phase was transferred to brown glass tubes and evaporated to dryness at $50\,^{\circ}C$ under

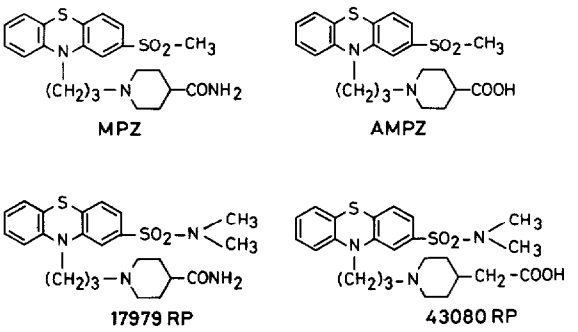

Fig 1 Structures of metopimazine (MPZ), its acid metabolite (AMPZ) and their internal standards (17979 RP and 43080 RP, respectively)

nitrogen. The residue was dissolved in 200 μl of mobile phase by whirlmixing for 5 s, and 30 μl were analysed by HPLC. Quantitation was by peak-height ratio with reference to the graphs obtained by analysing serum standards simultaneously

RESULTS

Evaluation of the analytical procedure

Fig. 2 shows chromatograms obtained from serum analyses, with both fluorescence and UV detection The insufficient sensitivity and selectivity of the UV detector is obvious. Plots of the standard curves of MPZ over the range 5–200 ng/ml and AMPZ over the range 10–500 ng/ml were linear. The line of best fit for MPZ was $y=1.69x+1.95$ ($n=16$, $r=1.00$) and for AMPZ $y=1.08x-12\ 00$ ($n=16$, $r=1\ 00$), where x is the analyte concentration and y the peak-height ratio The lower limits of detection for MPZ and AMPZ were 5 and 10 ng/ml (12 and 24 nmol/l), respectively The reproducibility was determined using spiked serum samples analysed at random on different days These spiked samples were stored at $-20\,^{\circ}C$ from one day to four months. The satisfactory reproducibility shown in Table I demonstrates that MPZ and AMPZ are stable for several months under the conditions mentioned above

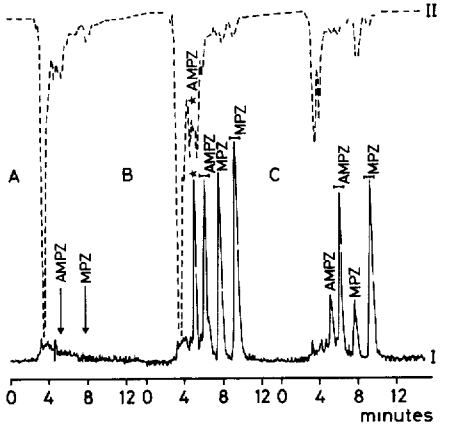

Fig 2 Chromatograms of human serum extracts (A) Blank serum analysed without internal standards, (B) serum to which metopimazine and its acid metabolite were added (100 and 250 ng/ml, respectively), (C) serum from a patient therapeutically treated with 3×60 mg metopimazine for 12 h The concentrations of metopimazine and its acid metabolite were determined as 40 and 106 ng/ml, respectively Peaks MPZ=metopimazine, AMPZ=the acid metabolite, I_{MPZ}=internal standard for MPZ, I_{AMPZ}=internal standard for AMPZ Curve I fluorescence detection, 280 nm excitation and 500 nm emission, sensitivity 2, time constant 3 s Curve II UV detection (254 nm), sensitivity 0 04 a u f s

TABLE I

REPRODUCIBILITY OF REPLICATE ANALYSES OF METOPIMAZINE AND ITS ACID METABOLITE ADDED TO HUMAN SERUM

Compound	Serum concentration (ng/ml)		Coefficient of variation (%)
	Added	Measured[a]	
MPZ	8 0	8 0	7 2
	20 0	20 3	6 8
	30 0	29 0	5 3
	40 0	38 9	3 5
	100 0	109 6	3 1
AMPZ	20 0	19 9	8 4
	50 0	49 4	6 0
	75 0	71 3	1 9
	100 0	100 0	3 1
	250 0	250 9	3 9

[a]Mean values from seven duplicate samples of each concentration

TABLE II

RELATIVE RETENTION TIMES OF MPZ, AMPZ, INTERNAL STANDARDS AND POTENTIALLY INTERFERING DRUGS

Compound	Relative retention time
MPZ	0 82
AMPZ	0 55
Internal standard for MPZ (17979 RP)	1 00
Internal standard for AMPZ (43080 RP)	0 66
Chlordiazepoxide	0 50
Flupenthixol	0 89
Chlorprothixene	0 80

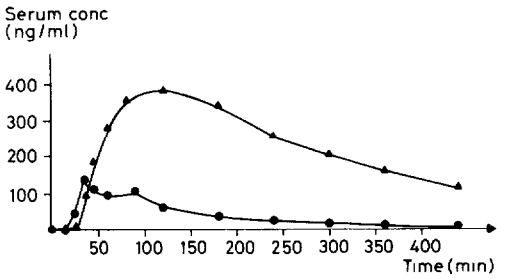

Fig 3 Concentrations of (●) metopimazine and (▲) its acid metabolite in serum from a patient treated with 50 mg of metopimazine given orally at time 0

Selectivity

Serum blanks from fifty drug-free patient samples did not show any interference from endogenous compounds, neither did those from patients undergoing treatment with the following cytostatics: cisplatin, teniposide, fluorouracil, doxorubicin, vindesine, cyclophosphamide, vincristine, methotrexate, etoposide and altretamine. The following drugs were investigated for possible interfering peaks in the same region as MPZ, AMPZ and their internal standards: codeine, chlordiazepoxide, chlorpromazine, chlorprothixene, dextropropoxyphene, diazepam, flupenthixol, ibuprofen, ketobemidone, levomepromazine, metoclopramide, morphine, nitrazepam, nordextropropoxyphene, oxazepam, paracetamol and salicylic acid. Table II shows the relative retention times of MPZ, AMPZ, the internal standards and the drugs that might interfere in the analysis.

Serum level study

This method has been used for the measurement of MPZ and AMPZ in a pharmacokinetic study [2]. An example of the serum levels in a patient receiving 50 mg of MPZ orally is shown in Fig. 3

DISCUSSION

The cyano-bonded phase provides good selectivity for separating many basic compounds and their metabolites [3–5]. Ammonium acetate has been used as buffer in the mobile phase for the separation of phenothiazines and related compounds [4]. We found this buffer suitable also for the separation of MPZ, AMPZ and their internal standards.

Acidified serum and a chlorinated organic solvent were necessary for the simultaneous extraction of MPZ and AMPZ, presumably because of the use of the ion-pair principle. Sufficient selectivity and sensitivity were obtained by means of a fluorescence detector (Fig. 2), which is more stable than the electrochemical detectors most often used for this purpose [4,5]

By comparing peak heights of MPZ and AMPZ from serum extracts with peak heights obtained by injection of a solution of equivalent amounts, recoveries of ca 80 and 25% were found for MPZ and AMPZ, respectively Despite the poor recovery of AMPZ adequate precision was obtained, probably due to the co-extracted chemically similar internal standard.

MPZ and AMPZ appeared to be fairly stable in both blood and serum samples. Patient blood samples left for 4 h at room temperature or for three days at 4 °C showed the same results as those immediately spun down to separate the serum. The patient serum samples were found to be stable at −20 °C for at least four months but after one year a significant decrease of MPZ was found (25%)

The described method is relatively simple and robust and has been per-

formed without trouble by different technicians during the past three years for the determination of serum levels in patients therapeutically treated with MPZ.

ACKNOWLEDGEMENTS

This work was supported by Rhône-Poulenc Pharma Norden A/S. We thank Jørgen Andersen, Merete Glaring and Karin Helene Nielsen for skilful technical assistance and Armand Frydman, Rhône-Poulenc (Antony, France) for providing the unpublished analytical method report and the standard materials.

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