Analysis of Hydroxyurea in Capsules and Aqueous Solution and Stability Study with Capillary Gas Chromatography and Thermionic (N–P) Specific Detection

Adnan El-Yazigi[1,2] and Sameer Al-Rawithi[1]

Received December 12, 1990; accepted July 2, 1991

A method for the analysis of hydroxyurea (HU) in solutions, powder, or capsules by use of capillary gas chromatography with N–P thermionic specific detection is described. Upon injection of an HU solution in a methanol and acetone mixture, the drug formed pyridine which was well separated from the internal standard (thiotepa) on a 30-m fused-silica, SE-30 capillary column with temperature programming. The peak height ratio versus concentration standard curves were linear with correlation coefficient ranging between 0.9942 and 0.9993. The coefficients of variation at 5, 25, and 50 μg/L were 7.2, 5.7, and 5.5%, respectively. Hydroxyurea was extracted from powder or capsule formulations with a mixture of methanol and acetone (50:50, v:v), and the percentage found of the label claim for 10 capsules ranged between 96.7 and 104.9 (mean = 100.1; CV = 2.7%). Further, this assay was used to examine the stability of hydroxyurea in aqueous solutions at 4, 23, and 45°C, and the apparent first-order rate constants observed at these temperatures were 0.06407, 0.08113, and 0.1293 day^{-1}, respectively; the activation energy was 3011 $cal \cdot K^{-1} \cdot mol^{-1}$.

KEY WORDS: capillary gas chromatography; analysis; stability; urea derivative; capsules; mass spectrometry.

INTRODUCTION

Hydroxyurea (HU) is an antineoplastic agent with cytotoxic activity exhibited in the S-phase through the inhibition of the ribonucleotide reductase involved in the synthesis of DNA (1,2). This drug has long been used in the treatment of chronic granulocytic leukemia and occasionally in the chemotherapy of melanoma and other solid malignancies (1,3).

The analysis of HU has been limited to either iodometric titration (United States and British Pharmacopoeias) or colorimetry (3–5). More recently, a high-performance liquid chromatographic method has been described for this agent (6). The use of gas chromatography has not been previously addressed; however, because of its sensitivity, accuracy, and high efficiency, this technique could provide an excellent tool for analysis of HU. In this report, we describe an expedient and accurate, indirect capillary gas chromatographic assay for HU in capsules and solutions and apply it to study the stability of this drug in aqueous solutions at different temperatures.

[1] Pharmacokinetics Laboratory, Pharmacology and Toxicology Section, Department of Biological and Medical Research, King Faisal Specialist Hospital and Research Center, Riyadh 11211, Saudi Arabia.

[2] To whom correspondence should be addressed.

MATERIALS AND METHODS

Materials

Analytical samples of hydroxyurea and thiotepa (IS) (internal standard) were supplied by Aldrich Chemical Co. (Milwaukee, WI) and Lederle Laboratories (American Cyanamid Co., Pearl River, NY), respectively. Methanol and acetone (Fisher Scientific Co., Fair Lawn, NJ) were HPLC grade.

Pharmaceutical Formulations

We analyzed HU in 10 of its 500-mg capsules (Hydrea, E. R. Squibb & Sons Ltd., London, England) obtained from a local hospital pharmacy.

Instrumentation

We used for this work a gas chromatograph (Model Vista 6000) (Varian Co., Palo Alto, CA) equipped with a thermionic nitrogen–phosphorus-specific detector, a split/splitless capillary injector, and a data module (Model Vista 402). The chromatographic separation was achieved by use of a 0.254-mm-I.D., 30-m fused-silica, SE-30 capillary column (Varian Co., Palo Alto, CA) and temperature programming. The oven temperature was initially held at 30°C for 5 min, then raised at a rate of 50°C/min to 220°C, which was held for 6 min. To cool the oven rapidly between runs down to the initial temperature, the door was manually opened. Nitrogen was used as a carrier gas (flow rate = 2.5 ml/min), and the detector gases were hydrogen and air at flow rates of 4 and 175 ml/min, respectively. The injection port and detector temperatures were 225 and 300°C, respectively.

Gas Chromatography–Mass Spectrometry (GC-MS)

GC-MS was used only to identify the peak observed following the injection of HU solution in equal portions of acetone-methanol. The instrument consisted of a gas chromatograph (Model 5890A), a mass spectrometer (Model 5970 MSD), and a data acquisition and library search computer (Model 59824A Scanning Interface with Model HP Vectra ES/12 PC), all from Hewlet-Packard (Palo Alto, CA). For this task, we used the same capillary column mentioned above with helium as a carrier gas (flow rate = 10 ml/min), and the mass spectrometer was operated in the electron impact mode with electron energy of 70 eV.

Calibration Curves

The linearity of the assay was examined by constructing on different days standard curves in the concentration range 5 to 400 μg/ml and determining the coefficients of correlation. To prepare such curves, transfer different amounts of HU to clean tubes, add 100 μl of 15 μg/ml of the internal standard to each tube, and bring the total volume of the sample to 1 ml with a methanol–acetone mixture (1:1, v:v).

0724-8741/92/0100-0115$06.50/0

Inject 1 to 2 µl of each solution, and plot the peak height ratio against the concentration of HU.

Precision

The precision was investigated by analyzing replicate samples of the following concentrations: 5, 25, and 50 µg/ml of HU in the above methanol–acetone mixture. To each sample, 100 µl of 15 µg/ml of the internal standard was added, the volume adjusted to 1 ml, and the analysis completed as described above.

Analysis of HU in Capsules

The capsule was carefully opened and the shell and content were both placed into a volumetric flask containing 1 liter of the methanol–acetone mixture. This rather large volume was found necessary to ensure a rapid and complete dissolution. After the flask was tightly sealed, the liquid was thoroughly stirred for 45 min using a magnetic stirrer and a portion was filtered through a 0.22-µm filter unit (Millipore Co., Bedford, MA). Duplicate samples of the filtrate were taken quickly and analyzed as described above after the addition of the appropriate amount of internal standard. The concentration of HU was calculated by use of standard curve prepared under similar conditions. Extreme caution was exercised to avoid solvent evaporation throughout the entire procedure. Ten capsules of Hydrea were analyzed according to this procedure.

Recovery of HU from Powder

The content of two batches of HU (five capsules each) was emptied into two bulk powders (Powder-I and Powder-II), and six accurately weighed aliquots of each of these well-mixed powders (i.e., 45 mg of Powder-I and 40 mg of Powder-II) were transferred to 100-ml volumetric flasks. To all but one of the flasks which received the 45-mg aliquot, a 5-mg aliquot of pure HU was added, and to all but one of the flasks which received the 40-mg aliquot, a 10-mg aliquot of pure HU was added. The sixth flask of each group did not receive the additional aliquot of pure HU and served as a blank. The volume was brought to the mark with a methanol–acetone (1:1, v:v) mixture containing 1.5 µg/ml of the internal standard. The liquid in each flask was filtered and analyzed as described above, and the amount recovered was calculated as the total amount found *minus* the amount found in the blank.

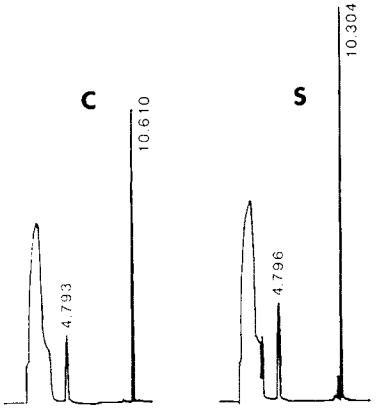

Fig. 1. Representative capillary gas chromatograms of an extract of a capsule content (C) and a standard solution (S) of HU in methanol–acetone (1:1, v:v) containing 1.5 µg/ml of internal standard. Retention time of HU = 4.793–4.796 min and that of the internal standard = 10.516–10.61 min.

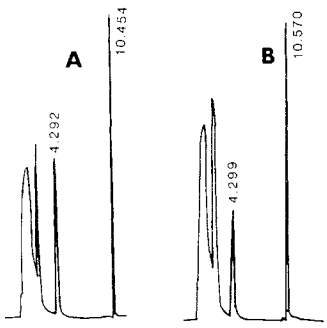

Fig. 2. Capillary gas chromatograms of a standard solution containing initially 50 µg/ml of HU in water and stored for 2 days (A) and 9 days (B) at 23°C. Prior to chromatography, the residues of fractions of this solution were redissolved in methanol–acetone (1:1, v:v) containing 1.5 µg/ml of internal standard. Retention time of 1 = 4.292–4.299 min and that of the internal standard = 10.454–10.57 min.

Stability Study

The stability of HU in aqueous solutions at concentrations equivalent to 10 and 50 µg/ml was studied at 4, 23, and 45°C. In these experiments, stock solutions of 10 and 50 µg/ml in water were prepared and 1-ml aliquots of each of these solutions were transferred to small tubes with tight caps. The tubes were divided into three groups each stored at one of the above temperatures. On different days and at the same time of the day, 100-µl aliquots from two tubes from each group were dried under a gentle stream of nitrogen gas, and the residue in each tube was redissolved in 100 µl of the methanol–acetone mixture which contains 0.15 µg of the internal standard. One to two microliters of each of these solutions was injected in the column. The concentrations were calculated using calibration curves constructed daily from fresh stock solutions prepared immediately prior to each experiment.

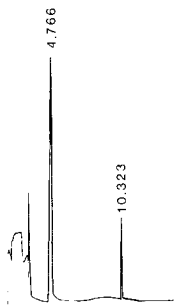

Fig. 3. A representative capillary gas chromatogram of an extract of a powder of capsules of HU in methanol–acetone (1:1, v:v) containing 1.5 µg/ml of internal standard. Retention time of HU = 4.766 min and that of the internal standard = 10.323 min.

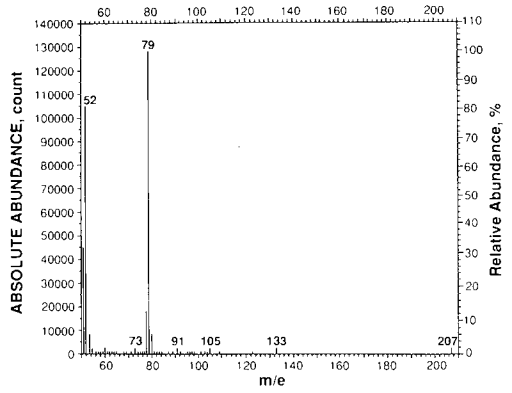

Fig. 4. Mass spectrum of the pyrolytic derivative of HU whose peak is observed after injection of pure HU solution in equal portions of acetone-methanol into the chromatograph. On-line computer spectra library search identified this spectrum to be that of pyridine.

RESULTS AND DISCUSSION

We present a gas chromatographic assay of HU which does not require a separate derivatization step. Further, it does not suffer from the low retention capacity (estimated k' = 0.35) and low detection wavelength (i.e., 214 nm) inherent with the HPLC method reported earlier for HU (6).

As shown in Figs. 1, 2, and 3, the peaks observed for both compounds were sharp as a result of the high chromatographic efficiency achieved with capillary gas chromatography. However, since urea compounds decompose upon heating (i.e., injection temperature = 225°C) and the peak observed for HU was eluted at 30°C oven temperature, while its melting point is 133–136°C, the eluting peak was expected to be a pyrolytic HU derivative which was subsequently shown by GC-MS to be pyridine (Fig. 4). Injecting a combined solution of HU and pyridine yielded a single peak with a retention time equal to that observed for HU alone. It is noteworthy that injection of other urea compounds [i.e., urea, biurea, thiourea, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU)] either did not yield any peak or yielded peaks with retention times quite distant from that observed for HU or pyridine. The reproducibility of the retention time of this peak is greatly enhanced (i.e., CV <1%) if the oven is allowed to steadily reach a temperature equilibrium at 30°C before an injection is made.

The ratio of the peak heights observed increased linearly with the concentration of HU in the range 10–400 μg/ml, and the correlation coefficient ranged from 0.9942 to 0.9993 (mean = 0.997; n = 19). The intercept of the standard curve was negative (i.e. −0.0595), indicating again that the peak produced by HU is that of a degradation product (*viz.* pyridine). The accuracy of the assay was equally good, with deviations from known standard solutions of 5, 25, and 50 μg/ml ranging from 2.8 to 3.2% (Table I). The coefficient of variation at different concentrations found was <7.2, which signifies a good assay precision.

Table I. Precision and Accuracy of the Described Method

Concentration prepared (μg/ml)	Concentration found (μg/ml)	CV of concentration found (n = 6) (%)	Deviation from perfect accuracy (%)[a]
5	5.16	7.2	3.2
25	25.7	5.7	2.8
50	51.6	5.5	3.2

[a] Estimated as 100 (concentration prepared *minus* concentration found)/concentration prepared.

Table II. Recovery of Hydroxyurea from Bulk Powder

Sample No.	Amount added (mg)	Amount found (mg)	Percentage recovery
1	5	4.95	99.1
2	5	4.82	96.5
3	5	4.97	99.5
4	5	4.65	93.0
5	5	5.61	112.2
6	10	10.59	105.9
7	10	9.36	93.6
8	10	9.23	92.3
9	10	9.89	98.9
10	10	10.08	100.8
Mean			99.2
SD			6.2

The recovery of HU from powder of a capsule formulation was investigated by adding 5- or 10-mg aliquots of pure HU to portions of this powder and analyzing the mixture as described above (Table II). The use of a methanol–acetone mixture (1:1, v:v) for extraction of HU from powder yielded a complete recovery (mean = 99.2%, SD = 6.2%) with clean chromatograms (Fig. 3).

Table III presents the results of the analysis of the commercially available capsule formulation of HU according to the described procedure. The percentage of label claim for all 10 capsules ranged from 96.7 to 104.9, with a mean (and SD) of 100.1 (2.7). Thus, if the USP content uniformity requirement (100 ± 15%) is to be applied here, this formulation meets this requirement.

The stability of HU in aqueous solutions at concentra-

Table III. Results of Analysis of Commercial Hydroxyurea Capsules According to the Described Procedure

Capsule No.	Percentage of label claim
1	96.9
2	104.2
3	96.7
4	104.9
5	99.5
6	99.0
7	101.1
8	100.2
9	99.5
10	99.2
Mean	100.1
SD	2.7
Range	96.7–104.9

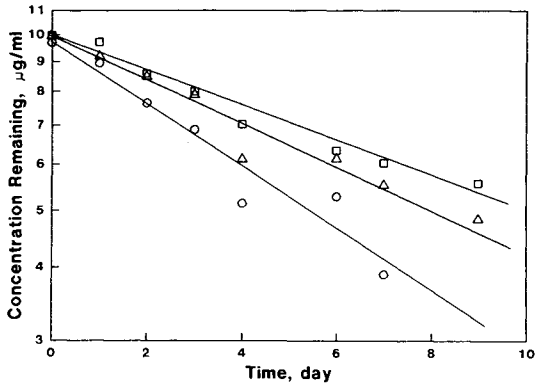

Fig. 5. Stability profiles of 10 μg/ml aqueous solution of HU at 4°C (□), 23°C (△), and 45°C (○).

tions equivalent to 10 and 50 μg/ml was investigated at 4, 23, and 45°C using the described assay. Figure 5 presents the stability profiles of HU at the lower concentration used, and similar profiles were obtained for the higher concentration. The data appear to follow the first-order kinetics, and the rate constants of HU degradation, estimated by the least-squares regression analysis, are presented in Table IV. There were no significant differences in the values of these constants between the two concentrations investigated ($P >$ 0.2 according to the paired t-test statistics); further, k_{obs} increased as a function of temperature. The data presented in Table IV were plotted according to the Arrhenius equation, and estimates of the activation energy for the degradation of HU were generated. The mean (and SD) value of this parameter was 3011 (623) cal · K^{-1} · mol^{-1}.

Table IV. The Apparent Rate Constants and Activation Energy for Degradation of Hydroxyurea in Aqueous Solutions

Concentration (μg/ml)	Degradation rate constant (day^{-1}) 4°C	23°C	45°C	Activation energy (cal · K^{-1} · mol^{-1})
10	0.06936	0.08505	0.1266	2,570
50	0.05878	0.07721	0.1319	3,451
Mean	0.06407	0.08113	0.1293	3,011
CV (%)	11.7	6.8	2.9	20.7

In conclusion, we present in this report a new indirect method for the analysis of hydroxyurea by capillary gas chromatography and thermionic specific (N-P) detector. This assay was used to analyze HU in capsules and to examine its stability in aqueous solution. The data generated indicate that HU is labile in water even if refrigerated.

ACKNOWLEDGMENTS

The authors wish to thank the Administration of the King Faisal Specialist Hospital and Research Centre for its support of the drug analysis and pharmacokinetics program in this institution. The assistance of Mr. Ali Al-Odaib in performing the GS-MS analysis is also appreciated.

REFERENCES

1. R. L. P. Adams and J. G. Lindsay. Hydroxyurea: Reversal of inhibition and use as a cel-synchronizing agent. *J. Biol. Chem.* 242:1314–1317 (1976).
2. C. W. Young, G. Schochetman, and D. A. Karnofsky. Hydroxyurea-induced inhibition of deoxyribonucleotide synthesis: Studies in intact cells. *Cancer Res.* 27:526–534 (1967).
3. R. J. Belt, C. D. Haas, J. Kennedy, and S. Taylor. Studies of hydroxyurea administered by continuous infusion. Toxicity, Pharmacokinetics, and cell synchronization. *Cancer* 46:455–462 (1980).
4. R. K. Sylvester, M. Lobell, M. E. Teresi, D. Brundage, and R. Dubowy. Excretion of hydroxyurea into milk. *Cancer* 60:2177–2178 (1987).
5. E. Fabricius and M. F. Rajewsky. Determination of hydroxyurea in mammalian tissues and blood. *Rev. Eur. Etudes Clin. Biol.* 16:679–683 (1971).
6. J. Pluscec and Y.-C. Yuan. High-performance liquid chromatographic analysis of hydroxyurea in pharmaceutical formulations and in the bulk. *J. Chromatogr.* 262:298–302 (1986).