Journal of Chromatography, 362 (1986) 298–302
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 699

Note

High-performance liquid chromatographic analysis of hydroxyurea in pharmaceutical formulations and in the bulk

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(Received April 3rd, 1986)

Hydroxyurea is structurally a simple compound that was first synthesized more than one hundred years ago[1]. However, its palliative role in the treatment of leukemia and in malignant melanoma was first recognized in 1963[2]. Hydrea (distributed in Germany as Litalir capsules) is the only commercially available pharmaceutical preparation of hydroxyurea.

The current analysis of hydroxyurea, as given in the United States Pharmacopoeia (U.S.P.), is an iodometric titration. Although an accurate method, it still lacks the simplicity of the high-performance liquid chromatography (HPLC) method. A number of HPLC methods have been described, however, they deal with the analysis of drugs and drug-related compounds that are derivatives of hydroxyurea[3–5]. Some of them require prior or post-column derivatization[6–8]. This paper describes a simple HPLC method for the determination of hydroxyurea and potential impurities which may be present in Hydrea or bulk.

EXPERIMENTAL

Apparatus

A Waters 6000 A pump and a Perkin-Elmer LC-55 variable-wavelength UV detector were used to perform the analyses. The UV detector was set at 214 nm with a range of 0.02 a.u.f.s. A supelco LC-18 Column (250 × 4.6 mm I.D.; particle size, 5 μm) was used for chromatographic separation with a Rheodyne 7010 injection valve fitted with a 20-μl sample loop for sample introduction and degassed distilled water as the mobile phase. A flow-rate of 0.5 ml/min was found to give acceptable retention times for hydroxyurea, possible impurities (hydroxylamine, urea) and the components present in the Hydrea formulation (citric acid, magnesium stearate, milk sugar). Chromatographic peaks were recorded on a Pharmacia recorder (chart speed 150 mm/h) and integrated by analog-to-digital converters in a Hewlett-Packard 3357 laboratory automatin system.

Reagents

The following reagents were used: hydroxyurea (reference standard or equivalent); Hydrea capsule (500 mg; E.R. Squibb and Sons); uracil (Eastman Kodak, Rochester, NY, U.S.A.); hydroxylamine hydrochloride (Fisher Scientific, Fairlawn, NJ, U.S.A.); and urea (J. T. Baker, Phillipsburg, NJ, U.S.A.).

0021-9673/86/$03.50

Internal standard solution

An accurately weighed amount of uracil (24–26 mg) was transferred into a 200-ml volumetric flask. About 150 ml of distilled water were added. The flask was placed in an ultrasonic bath (Sonicor Instrument Corporation, Copiague, NY, U.S.A.) until solution was complete. The solution was then diluted to volume and mixed.

Standard solution

Hydroxyurea reference standard (48–52 mg) was accurately weighed into a 50-ml volumetric flask. To the flask, 10 ml of the internal standard solution were added, and the resulting solution was diluted to volume with water.

Sample preparation of capsule

The average weight of the content of Hydrea capsules was determined from the difference in weights of 20 filled and empty capsules. The contents of 20 capsules was placed in a glass mortar and ground quickly to a fine, smooth powder. In duplicate, accurately weighed amounts (3.1–3.3 g) of powder were transferred quantitatively into 1000-ml volumetric flasks. To each of the flasks, 900 ml of distilled water were added and the contents were sonicated for 5 min. The solutions were then stirred on a magnetic stirrer for an additional 30 min at room temperature, diluted to volume with distilled water, mixed and sonicated for an additional 5 min. An aliquot of 75 ml from each solution was filtered through a membrane filter (Versapor 1200, supported membrane filter, 12 μm, Gelman Science, Ann Arbor, MI, U.S.A.) discarding the first 10–15 ml from each solution. Aliquots of 25 ml of the clear filtrate were transferred to 50-ml volumetric flasks. To each flask, 10 ml of the internal standard solution were added before the solutions were diluted to volume with distilled water and mixed.

Raw material sample preparation

It was carried out as described under *Standard solution.*

System suitability test

Resolution. The hydroxylamine peak is closest to the peak of hydroxyurea. These peaks were resolved completely (resolution > 1.0).

Precision. The standard solution was chromatographed repeatedly and the coefficient of variation (C.V.) calculated for the response ratios of hydroxyurea *versus* uracil. A precision with a C.V. of $\pm$ 0.6% was observed for five consecutive chromatographic runs.

Calibration. The linearity of response with respect to concentration was tested with standard concentrations from 20 to 140% of the labeled potency. The correlation coefficient was found to be 0.9999 with a slope of 1.255 and a Y-intercept of -0.01.

Sample resolution

Baseline resolution was achieved between the citric acid used in the formulation of Hydrea and the hydroxyurea (resolution = 1.8).

Recovery study

Placebos used in the formulation of Hydrea, and samples of Hydrea were spiked with hydroxyurea equivalent to 100% and 30% of label respectively. The average recovery was 100.5% (C.V. = 0.4%) for the placebos and 99.2% (C.V. = 2.6%) for samples. The average material balance of spiked samples was 99.8% (C.V. = 0.6%).

Assay

Aliquots (20 μl) of the standard and sample preparation of Hydrea and raw material were injected into the chromatograph. Chromatograms were recorded and the peak heights of hydroxyurea and uracil were measured. The concentration of the active ingredient, in mg per capsule, was calculated as follows:

$$\text{mg/caps} = \frac{R_u\ W_s\ C\ P\ 10}{R_s\ W_u\ 25}$$

where R_u = response ratio (hydroxyurea to uracil) of active ingredient in the sample preparation; R_s = response ratio (hydroxyurea to uracil) of active ingredient in the standard preparation; W_u = weight of the sample in mg; W_s = weight of the standard in mg; C = average capsule net content in mg/caps; P = purity of the standard.

RESULTS AND DISCUSSION

During the course of the study, a number of chromatographic columns was tested (PRP-1, Partisil ODS-2, cyano phases, ion-exchange materials etc); however, only the one described in this work gave a satisfactory separation of hydroxyurea from potential impurities resulting from decomposition. An infrequent impurity originating from the synthesis of hydroxyurea, misnamed isohydroxyurea (O-carbamoylhydroxylamine) is also well separated from hydroxyurea.

Complete baseline separation was achieved between hydroxyurea and hydroxylamine, the most frequently observed impurity, as noted in Fig. 1.

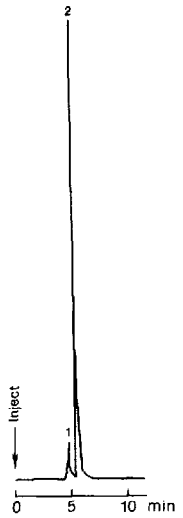
Analyses of a series of formulations are summarized in Table I with a typical chromatogram depicted in Fig. 2. All results are within regulatory limits (±3% of labeled) with an average of 99.1%. The analyses of five batches of hydroxyurea raw

TABLE I

RESULTS OF ANALYSIS OF HYDREA 500 mg

Each sample represents an average of 15 different weights and chromatographic runs.

Sample	*Hydroxyurea found*		*C.V. (%)*
	mg/caps	*% label*	
1	486	97.2	1.3
2	495	99.0	1.2
3	509	101.8	0.9
Mean	497	99.1	1.1



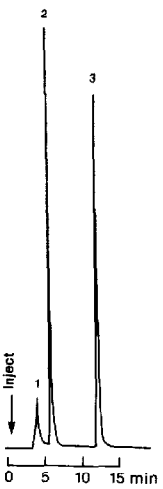


Fig. 1. Resolution of hydroxylamine (1) and hydroxyurea (2).

Fig. 2. Typical HPLC analysis of hydroxyurea in Hydrea capsule 500 mg. Peaks: 1 = citric acid present in the formulation of Hydrea; 2 = hydroxyurea; 3 = uracil (internal standard).

Fig. 3. HPLC analysis of hydroxyurea raw material. Peaks: 1 = hydroxyurea; 2 = uracil (internal standard).

TABLE II

RESULTS OF ANALYSIS OF HYDROXYUREA RAW MATERIAL

Each sample represents an average of four different weights and seven chromatographic runs.

Sample	*Found (%)*	*C.V. (%)*	*U.S.P. method (iodometric) (%)*
1	99.7	0.3	–
2	99.7	0.9	99.3
3	99.9	0.4	99.1
4	99.7	0.6	99.4
Mean	99.8	0.6	99.3

TABLE III

RECOVERY STUDY OF HYDROXYUREA

Data are average of six determinations.

Determined in sample (mg)*	*Added (mg)*	*Found (mg)*	*Material balance** (%)*	*C.V. (%)*	*Recovery**** (%)	C.V. (%)
2037	604.3	2636	99.8	0.6	99.1	2.6

* 3.1–3.3 g powder (corresponding to four capsules) obtained by grinding 20 capsules, was used for each determination.

** Material balance $= \frac{\text{found}}{\text{added} + \text{determined in sample}}$

*** Recovery $= \frac{\text{found} - \text{determined in sample}}{\text{added}}$

material were compared with data obtained by the iodometric analyses given in the U.S.P. (Table II). Although the results are comparable, the HPLC method described in this paper is faster, simpler and more reliable than the currently used U.S.P. method. A typical chromatographic run of the raw material is seen in Fig. 3. The recovery study is presented in Table III.

In summary, a fast, simple and accurate method for hydroxyurea in a pharmaceutical preparation and in a raw material is presented.

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