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Note

Stability-indicating high-performance liquid chromatographic method for the simultaneous determination of cisplatin and 5-fluorouracil in 0.9% sodium chloride for injection

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The serendipitous discovery by Rosenberg et al. [1] that cell replication could be inhibited by platinum complexes led to the development and therapeutic use of cisplatin (*cis*-diamminedichloroplatinum II) as an anticancer agent. Cisplatin is currently used in treating testicular, ovarian, and lung cancers as well as squamous cancers of the head and neck [2–5].

The anticancer agent 5-fluorouracil (5-FU) is currently used in treating several malignancies including cancers of the lung, head and neck, and colon [3,4]. Cisplatin and 5-FU are often given in combination to treat a variety of malignancies [5,6]. The admixture of more than one anticancer drug in a single intravenous solution is both cost-effective and advantageous to the patient. Combinations of drugs admixed in the same intravenous fluid can result in the precipitation or loss of potency of one or both drugs. Since questions often arise concerning the compatibility of cisplatin and 5-FU in the same intravenous admixture, a study of the stability and compatibility of the combination of cisplatin and 5-FU in 0.9% (w/v) sodium chloride solution was designed.

Loss of potency of one or more of the drugs in the intravenous admixture may occur without obvious visual changes. Therefore, a rapid, simple, and pre-

cise stability-indicating high-performance liquid chromatographic (HPLC) assay for the simultaneous determination of cisplatin and 5-FU in intravenous fluids was necessary.

Various methodologies have been used to quantitate cisplatin including atomic absorption spectrometry (for elemental platinum only) [7–9], ultraviolet (UV) spectrometry [10], and reversed-phase HPLC [11–16]. Cisplatin has poor retention characteristics in reversed-phase HPLC systems. Solvent-generated anion exchangers and ion-pairing agents have been used to improve retention, peak shape, and reproducibility [11–14]. Analysis of the fluoropyrimidines, including 5-FU has most commonly been through HPLC methodology [17]. Reversed-phase HPLC assays have utilized paired-ion chromatography [18–20] and gradient methods [21]. No published assay method for either cisplatin or 5-FU could be used as a stability-indicating assay for the simultaneous determination of cisplatin and 5-FU in intravenous fluids.

This report describes the development, validation, and application of a stability-indicating HPLC assay for cisplatin and 5-FU. This assay utilizes an ion-pairing agent (tetrabutylammonium hydroxide) and an isocratic solvent system to simultaneously determine cisplatin and 5-FU in commercial intravenous solutions.

EXPERIMENTAL

High-performance liquid chromatography

The instrumentation consisted of a Beckman (Fullerton, CA, U.S.A.) 110B pump, a Rheodyne (Cotati, CA, U.S.A.) 7125 injector fitted with a 10-μl loop, and a Beckman 163 variable-wavelength detector (313 nm). Detection outputs were computed with a Shimadzu (Columbia, MD, U.S.A.) C-R5A Chromatopac integrator. During specificity and stability studies, a Fisher Scientific (Pittsburgh, PA, U.S.A.) Recordall 5000 strip chart recorder was also utilized.

A PhaseSep Spherisorb ODS 1 (15 cm×4.6 mm) column packed with C_{18} bonded silica (5 μm particle size) was obtained from Rainin (Woburn, MA, U.S.A.). Column temperatures were at 24–27°C (ambient temperature). A flow-rate of 1.0 ml/min was used throughout the procedure.

Materials

Analytical-grade cisplatin was supplied by the Analytical Research and Development Department, Bristol-Meyers, Industrial Division (Syracuse, NY, U.S.A.). Analytical grade 5-FU was obtained from Sigma (St. Louis, MO, U.S.A.). Sodium chloride, 0.9% (w/v) for injection, was obtained from Travenol Laboratories (Deerfield, IL, U.S.A.). Tetrabutylammonium hydroxide (TBAH) 0.4 M titrant was obtained from Eastman Kodak (Rochester, NY, U.S.A.).

Mobile phase preparation

A 2% (v/v) TBAH solution in Millipore (Bedford, MA, U.S.A.) water was prepared and adjusted to pH 6.0 with concentrated orthophosphoric acid. The solution was filtered and degassed by ultrasonication for 5 min. Prior to analytical runs, the mobile phase was pumped through the column for 30 min to allow for equilibration.

Standard preparations

A 500 μg/ml cisplatin standard solution was prepared by weighing the appropriate amount of cisplatin and adding 0.9% (w/v) sodium chloride for injection. A 1000 μg/ml 5-FU standard solution was prepared by weighing the appropriate amount of 5-FU and adding 0.9% (w/v) sodium chloride for injection.

Linearity studies

A cisplatin stock solution of 800 μg/ml was prepared in 0.9% (w/v) sodium chloride for injection. Appropriate dilutions with 0.9% (w/v) sodium chloride were made to achieve final cisplatin concentrations of 100, 200, 500 and 800 μg/ml. Triplicate injections (10 μl) of each solution were made for each concentration prepared.

A 5-FU stock solution of 5000 μg/ml was prepared in 0.9% (w/v) sodium chloride for injection. Appropriate dilutions with 0.9% (w/v) sodium chloride for injection were made to achieve final 5-FU concentrations of 125, 150, 175, 200 and 500 μg/ml. Triplicate injections (10 μl) were made for each concentration prepared.

Precision and accuracy studies

Within-day precision. From the 500 μg/ml cisplatin stock solution and the 1000 μg/ml 5-FU solution, aliquots of the cisplatin and 5-FU solutions were placed into the same test tube. An appropriate amount of 0.9% (w/v) sodium chloride for injection was added to the tube to bring the final concentration to 200 μg/ml for both cisplatin and 5-FU. Ten replicate injections (10 μl) of the cisplatin–5-FU solution were made on the HPLC system on the same day.

Between-day precision. From the 500 μg/ml cisplatin standard solution and the 1000 μg/ml 5-FU standard solution, both prepared daily, aliquots from the cisplatin and 5-FU solutions and 0.9% (w/v) sodium chloride for injection were combined to achieve final concentrations of 200 μg/ml cisplatin and 200 μg/ml 5-FU. On each of five days, ten replicate injections of the cisplatin–5-FU solution were made on the HPLC system.

Chromatographic accuracy. A 200 μg/ml cisplatin and 5-FU [in 0.9% (w/v) sodium chloride for injection] control solution was prepared daily. Triplicate injections (10 μl) of this solution were made daily for five days. Individual peak

areas of the cisplatin and 5-FU concentrations were compared to mean peak areas obtained from cisplatin and 5-FU calibrators.

Cisplatin and 5-FU specificity

The purpose of this experiment was to determine if co-eluting peaks were present when cisplatin and 5-FU were analyzed simultaneously. Three solutions were prepared: 200 μg/ml cisplatin, 200 μg/ml cisplatin plus 1000 μg/ml 5-FU and 1000 μg/ml 5-FU. All solutions were prepared in 0.9% (w/v) sodium chloride for injection.

Two UV detectors were connected in series. Detector 1, set to 313 nm and attenuation of 4, was connected to a Shimadzu C-R5A Chromatopac integrator. Detector 2, set to 240 nm, was connected to a Fisher Scientific Recordall Series 5000 strip chart recorder. Detector 2 was adjusted to 2.0 a.u.f.s during analysis of 5-FU and 0.02 during analysis of cisplatin. Peak-height ratios were compared for cisplatin alone, 5-FU alone, and cisplatin and 5-FU in combination.

Stability-indicating studies

The purpose of these experiments was to determine if the assay was stability-indicating for cisplatin and 5-FU in the presence of potential degradation products. On day 1 a combined solution of 200 μg/ml cisplatin and 1000 μg/ml 5-FU was prepared in 0.9% (w/v) sodium chloride for injection. This solution was exposed to fluorescent light (85–95 footcandles) and stored at room temperature for 24 h. These conditions were known to result in cisplatin degradation [22]. The chromatographic system and conditions were the same as described in the specificity studies except as follows. The integrator connected to detector 1 was set to attenuation 4 during analysis of cisplatin and 5 during analysis of 5-FU. Detector 2 was adjusted to an a.u.f.s. setting of 0.005 during analysis of cisplatin and 2.0 during analysis of 5-FU. Six injections of this solution were made (three for analysis of cisplatin, three for analysis of 5-FU) at baseline (day 1) and six injections were made 24 h later. Previous studies in our laboratory demonstrated that at 24 h greater than 50% of cisplatin had degraded (data not shown).

On day 1 and 2, a 200 μg/ml cisplatin and 1000 μg/ml 5-FU reference standard solution was prepared. At baseline (day 1), six injections of the combined solution were made (three to determine cisplatin peak-height ratios and three to determine 5-FU peak-height ratios). Peak-height ratios were compared between the reference standard solutions and the cisplatin–5-FU solution exposed to light.

RESULTS AND DISCUSSION

Chromatograms of 200 μg/ml cisplatin, 200 μg/ml 5-FU and 200 μg/ml cisplatin–5-FU are shown in Fig. 1. The approximate retention times of cisplatin

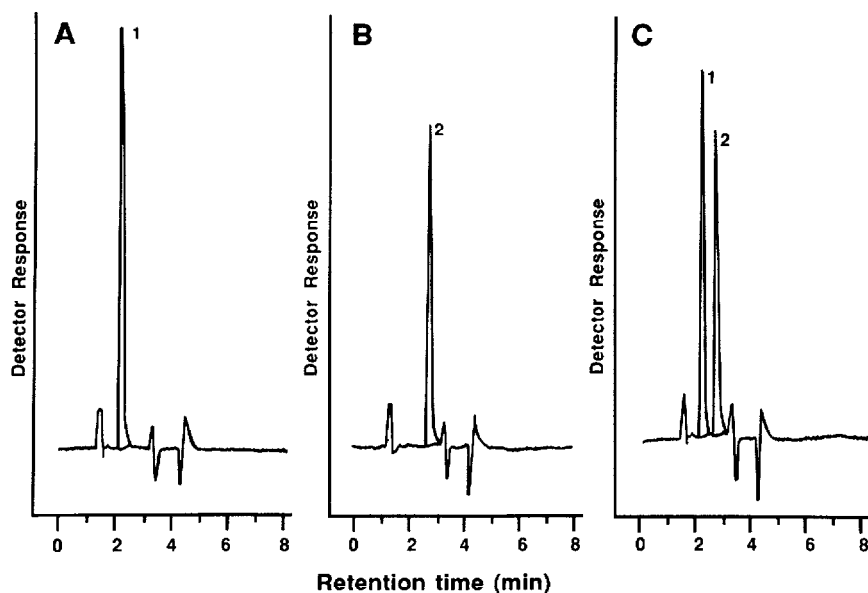Fig. 1. Chromatograms of 10 μl of 200 μg/ml cisplatin (A), 10 μl of 200 μg/ml 5-FU (B), and 10 μl of 200 μg/ml cisplatin–5-FU in 0.9% (w/v) sodium chloride for injection. Peaks: 1 = cisplatin; 2 = 5-FU. Approximate retention times: cisplatin, 2.1 min; 5-FU, 2.6 min Detector sensitivity is 1 mV full scale and the integrator is set at an attenuation of 16.

and 5-FU were 2.1 and 2.6 min, respectively (Fig. 1A and B). The retention times of the two drugs in combination (Fig. 1C) was observed to be approximately the same as each drug injected alone (Fig. 1A and B).

Linearity studies

A linear relationship was observed for peak area and concentration for both cisplatin and 5-FU over the concentrations evaluated (5-FU peak area $= 0.0211x - 0.0605$, correlation coefficient $= 0.999$; cisplatin peak area $= 0.0275x - 0.0525$, correlation coefficient $= 0.999$). Similar linearity studies conducted in our laboratory with concentrations of 5-FU greater than 1000 μg/ml have shown that 5-FU detector response is non-linear. Based on our data, we suggest that determination of 5-FU concentrations greater than 500 μg/ml necessitates the use of multiple-point calibration curves rather than single-point calibration due to the non-linearity of 5-FU detector response.

Precision and accuracy studies

The within-day coefficient of variation (C.V.) for the 200 μg/ml cisplatin and 5-FU calibrators were 1.1 and 3.2%, respectively (Table I). Within-day accuracy values for the 200 μg/ml cisplatin and 5-FU controls were 98.6 and 101.5% of the target and 5-FU calibrator concentrations (Table I).

TABLE I

PRECISION AND ACCURACY FOR CISPLATIN AND 5-FU

	Peak area (mean ± S.D.)	C.V. (%)	Accuracy (%)
Cisplatin (200 µg/ml)			
Within-day	50 172 ± 530	1.1	98.6
Between-day	50 843 ± 1089	2.1	100.6
5-Fluorouracil (200 µg/ml)			
Within-day	37 717 ± 1240	3.2	101.5
Between-day	37 281 ± 1386	3.7	100.9

TABLE II

SUMMARY OF CISPLATIN–5-FU SPECIFICITY STUDIES

Compound	Peak-height ratio[a] (mean ± S.D., $n=3$)	Difference[b] (%)
Cisplatin		
200 µg/ml reference standard	1.879 ± 0.0106	
200 µg/ml in cisplatin–5-FU combined solution	1.875 ± 0.0	0.186
5-Fluorouracil		
1000 µg/ml reference standard	0.699 ± 0.0035	
1000 µg/ml in cisplatin–5-FU combined solution	0.707 ± 0.0007	1.132

[a]Determined by dividing the peak height (cm) at 313 nm by the peak height at 240 nm.
[b]Difference = (peak-height ratio$_{ref}$ − peak-height ratio$_{comb}$)/peak-height ratio$_{ref}$ × 100%.

Between-day coefficients of variation for the 200 µg/ml cisplatin and 5-FU calibrators were 2.1 and 3.7%, respectively (Table I). Between-day accuracy values for the 200 µg/ml cisplatin and 5-FU controls were 100.6 and 100.9% of the target cisplatin and 5-FU calibrator concentrations (Table I).

Cisplatin and 5-FU specificity

The results of our specificity studies indicate that when analyzed simultaneously, the cisplatin and 5-FU peaks do not co-elute. Peak-height ratios for both cisplatin and 5-FU (injected alone) compared to the cisplatin and 5-FU peak-height ratios (injected in combination) were not different (Table II). These data suggest that no interfering peaks can be detected when cisplatin and 5-FU are analyzed simultaneously.

Stability-indicating studies

After allowing the cisplatin–5-FU solution to stand exposed to light for 24 h, a greater than 50% decrease in the cisplatin peak area (concentration) was observed (no decrease in 5-FU peak area/concentration was observed). As is demonstrated in Table III, the baseline peak-height ratios for the cisplatin–5-FU solution were approximately the same for the cisplatin–5-FU reference standard solution (difference for cisplatin and 5-FU was 0.6 and 1.42%, respectively).

Peak-height ratios for the cisplatin–5-FU solution exposed to light for 24 h were approximately the same for the cisplatin–5-FU reference standard solution. The percentage difference in the peak-height ratios of cisplatin and 5-FU compared to the cisplatin–5-FU reference standard solution was approximately the same (difference for cisplatin and 5-FU was 0.23 and 0.20%, respectively). Even after greater than 50% degradation of cisplatin, the peak-height ratios of the degraded solution and the freshly prepared solutions are approximately the same indicating that this assay is capable of analyzing cisplatin and 5-FU even after substantial degradation of cisplatin has occurred. No evidence is present to suggest that the degradation products co-elute with either cisplatin or 5-FU, thus indicating the assay to be stability-indicating.

Quantitation of cisplatin and 5-FU has been achieved by a variety of analytical techniques [7–21]. Although several elaborate reversed-phase HPLC methods have been reported for determining the stability of either cisplatin or 5-FU alone [16,23–25] no published methods were available to simultaneously determine the stability and compatibility of cisplatin and 5-FU in intravenous

TABLE III

SUMMARY OF CISPLATIN-5-FU STABILITY-INDICATING STUDIES

Compound	Day	Peak-height ratio[a] (mean ± S.D.)	Difference[b] (%)
Cisplatin (200 μg/ml)	1		
Reference standard		0.468 ± 0.0044	
Cisplatin–5-FU admixture		0.465 ± 0.0064	0.6
Cisplatin (200 μg/ml)	2		
Reference standard		0.445 ± 0.0014	
Cisplatin–5-FU admixture		0.446 ± 0.0049	0.23
5-Fluorouracil (1000 μg/ml)	1		
Reference standard		0.494 ± 0.0060	
Cisplatin–5-FU admixture		0.487 ± 0.0069	1.42
5-Fluorouracil (1000 μg/ml)	2		
Reference standard		0.417 ± 0.0053	
Cisplatin–5-FU admixture		0.416 ± 0.0030	0.20

[a]Determined by dividing the peak height (cm) at 313 nm by peak height at 240 nm.
[b]Difference = (peak-height ratio$_{ref}$ − peak-height ratio$_{comb}$)/peak-height ratio$_{ref}$ × 100%.

admixtures. The analytical procedure described above represents a simple, precise, and rapid method of simultaneously determining cisplatin and 5-FU concentrations in 0.9% (w/v) sodium chloride for injection. Currently the application of this assay to determine the stability and compatibility of cisplatin in the presence of other drugs in intravenous admixtures is in progress. In addition, an application of this assay to quantitative cisplatin concentrations in human plasma samples is being investigated in our laboratory. Due to the simple nature of this HPLC assay, it could have potential for widespread use in the biomedical community.

ACKNOWLEDGEMENTS

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