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Development of an HPLC Method for Simultaneous Analysis of Five Antineoplastic Agents

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Simultaneous analysis of common antineoplastic agents potentially hazardous to healthcare workers is of much interest for the evaluation of the overall health risk to these workers. Such analysis could be applied to both air and surface monitoring samples to provide a broader indication of risk to combinations of these agents. It was determined that the ability to simultaneously evaluate five frequently used, potentially hazardous agents was sufficient for general evaluation of exposures to healthcare workers. The approach used to select the five agents was to obtain a list of the agents used most frequently in both a cancer hospital and an outpatient cancer treatment center, then review the list to determine which agents were potentially more hazardous to human health. From these reviews, it was decided to attempt to develop an analytical method able to detect and quantify the presence of 5-fluorouracil, ifosfamide, cyclophosphamide, doxorubicin HCl, and paclitaxel. A reverse-phase high performance liquid chromatograph (HPLC) with a Waters Symmetry C8 column and a UV wavelength of 195 nm was selected for method development. The mobile phase was 22.75 percent acetonitrile in water buffered to a pH of 6.0. The HPLC analytical method developed is able to detect all five agents of interest, and at minimum detectable concentrations of 0.5-μg/mL for each of the five agents.

Keywords Antineoplastics, HPLC Analysis, Fluorouracil, Ifosfamide, Cyclophosphamide, Doxorubicin, Paclitaxel

A variety of antineoplastic agents are used in both hospital and outpatient cancer treatment centers. Many of these agents are potentially hazardous to the health of those healthcare workers involved in preparation and administration of these agents (e.g., oncology pharmacists and nurses),[(1)] as well as those involved in care of cancer patients (e.g., nurses, housekeepers, janitors, etc.). It is therefore important to have accurate methods for evaluation of the exposures received by these individuals from such agents. This requires an accurate and sensitive analytical method to detect concentrations of such agents at very low concentrations.

Currently, acceptable analytical methods do exist for several of these antineoplastics, but usually only for an individual agent or for small groups of chemically similar agents.[(2–5)] However, this method was developed to provide analytical detection for a wider range of the types of agents used, and with a single analysis. This capability could, for example, provide more information on exposures to health care workers from such agents.

The HPLC method is most often referred to in current literature on analytical methods for determination of antineoplastic agents.[(2–5)] This method appears to be most feasible for attaining the maximum sensitivity (lower limit of detection) when used for detection of multiple antineoplastics in both air and surface samples. As a result of this information, HPLC was selected for the method to be developed.

Thus, the major objective associated with this project was the enhancement of an existing HPLC method of analysis for cyclophosphamide to improve sensitivity and include accurate and sensitive detection and quantification for four other antineoplastic agents; ifosfamide, 5-fluorouracil, doxorubicin, and paclitaxel.

It was decided that the ability to simultaneously evaluate five agents was sufficient for general exposure evaluation. The approach used to decide which agents to include in this analytical method began by first obtaining a list of those agents most frequently used in both a cancer hospital and in an outpatient cancer treatment center. The list of agents commonly used by both was then reviewed to determine which agents were potentially more hazardous to human health. From this review of agents it was decided to attempt to develop an analytical method able to acceptably detect and quantify the presence of cyclophosphamide, ifosfamide, 5-fluorouracil, doxorubicin HCl, and paclitaxel.

Many of these agents are cytostatic drugs that have pharmacological properties linked with potential genotoxicological

TABLE I
Physical characteristics of antineoplastics[A]

Antineoplastic & formula	Molecular weight	Melting point	Soluble in
5-Fluorouracil $C_4H_3FN_2O_2$	130.1	282°C	Water
Ifosfamide $C_7H_{15}Cl_2N_2O_2P$	261.1	48°C	Water, saline, or methanol
Cyclophosphamide $C_7H_{15}Cl_2N_2O_2$ P·H_2O	279.1	45°C	Water, saline, or methanol
Doxorubicin HCl $C_{27}H_{29}NO_{11}$·HCl	580.0	209°C	Water
Paclitaxel $C_{47}H_{51}NO_{14}$	853.9	217°C	Methanol (highly lipophilic)

[A]From manufacturers' data sheets.[18,19,31–33,36]

hazards. Because the mechanisms of interaction of these drugs frequently involve interaction with DNA, RNA, or protein synthesis, many are known to have carcinogenic or mutagenic effects.[6–16]

Cyclophosphamide is an alkylating agent. It is classified by the International Agency for Research on Cancer (IARC) as both animal and human carcinogen, mutagen, and teratogen.[15,17–28] Cyclophosphamide is the drug of choice for the treatment of many types of cancer (e.g., Hodgkin's disease, multiple myeloma, a variety of leukemias, adenocarcinoma of the ovary, lymphomas, and breast cancer) and is an essential component of many effective drug combinations.[18–20,28,29]

Ifosfamide is a structural isomer of cyclophosphamide, but there are substantial differences in the spectrum of antitumor activity and toxicity between ifosfamide and cyclophosphamide.[20,30,31] Ifosfamide is also an alkylating agent and has been shown to be carcinogenic in rats, with female rats showing a significant incidence of leiomyosarcomas (a benign tumor) and mammary fibroadenomas. The mutagenic potential of ifosfamide has been documented in bacterial systems in vitro and mammalian cells in vivo.

5-Fluorouracil is a fluorinated pyrimidine and an antimetabolite.[32] It interferes with the synthesis of deoxyribonucleic acid (DNA) and inhibits formation of ribonucleic acid (RNA). The effects are most marked in those cells that grow more rapidly and that take up fluorouracil at a more rapid rate such as in a fetus, as well as in a cancer. For this reason, 5-FU falls in Pregnancy category D, Teratogenic effects risk.

Doxorubicin HCl, also known as adriamycin, is a cytotoxic anthracycline antibiotic isolated from cultures of the fungus *Streptomyces peucetius* var. *caesius*.[33] The most common uses for doxorubicin in cancer therapy is for various types of testicular cancer, leukemia, Ewing's sarcoma, Hodgkin's disease, and Kaposi's sarcoma.[33,34] Doxorubicin has been shown to have mutagenic and carcinogenic properties in experimental models.

Paclitaxel, a diterpene amide, is an antimicrotubule agent and was first isolated from the bark of the Pacific Yew (*Taxus brevifolia*) and is the primary component of taxol, the commercially available agent.[35,36] Therapeutically, taxol is of significant importance for treatment of ovarian cancer, breast cancer, carcinoma of the lungs, and head and neck carcinoma. The major toxicity is bone marrow depression with neutropenia, the common dose-limiting toxicity. Taxol can cause fetal harm when administered to a pregnant woman.

Based on the health hazard information for the five agents of interest, an emphasis was placed on cyclophosphamide for developing an acceptable analytical method. This was because it is a known human carcinogen (IARC) and because it has a high frequency of use and in relatively high concentrations.

A review of the physical characteristics of each antineoplastic of interest (fluorouracil, ifosfamide, cyclophosphamide, doxorubicin, and paclitaxel) was conducted (see Table I), followed by a review of existing analytical methods. Results from these reviews found a significant difference in chemical structures, and chemical and physical characteristics between most agents, along with a variety of analytical methods having been developed for their individual detection. Methods included gas chromatography (GC),[2] gas chromatography–mass spectroscopy (GC-MS),[37] gas chromatography–tandem mass spectroscopy (GC-MS-MS),[3] and reverse-phase high performance liquid chromatography (HPLC).[2,24,28,38–43] The most frequently used was the reverse-phase HPLC. Therefore, a reverse-phase HPLC system was chosen for analytical method development.

METHODS

Analytical Equipment

The analytical equipment used for developing this method was a BIO-RAD MAPS 100 (Monoclonal Antibody Purification System) Preparative System, with a programmable UV/VIS monitor. This is a reverse-phase HPLC unit equipped with a precision flow pump; an auto sampler with an injection loop of 500 μL; and a UV-VIS detector. Because of an irreparable failure with the MAPS 100 HPLC unit, the stability tests were conducted on a newer, BIORAD HPLC w/Model 1790 Programmable UV/VIS monitor.

This newer reverse-phase HPLC unit was also equipped with a precision flow pump; an auto sampler with an injection loop of 500 μL; and a UV-VIS detector. Sensitivity (AUFS) for this instrument was set at 0.05 for four of the agents, with 5-fluorouracil having an AUFS setting of 0.4. This instrument proved to have much greater sensitivity than the MAPS 100, thus the significant difference in the peak heights for the same agents tested at the same concentrations.

UV Detection

Of initial importance was determination of an acceptable wavelength setting for the UV detector. Because of the significant differences between most of the agents, it was important to ensure a common UV range existed to assure simultaneous detection.

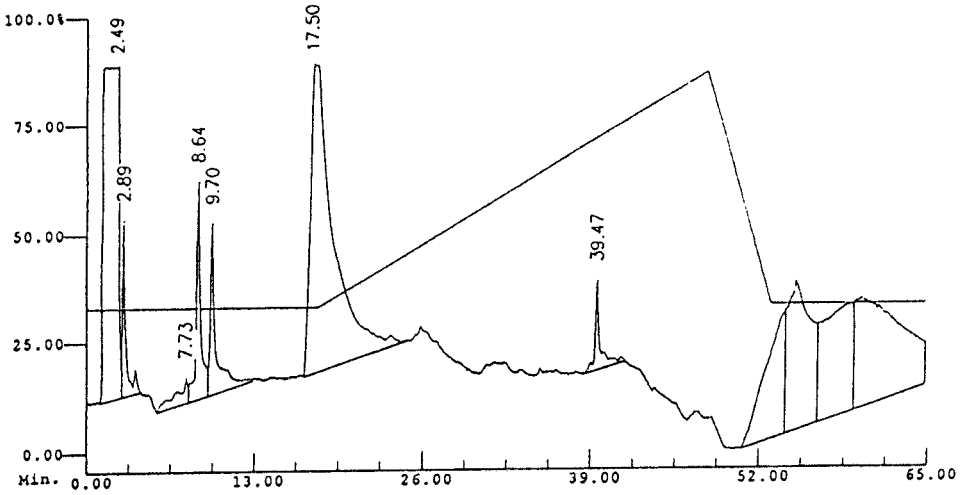
In the literature, a number of different UV wavelengths have been used in past and existing analytical methods for the agents of interest. The wavelengths ranged from 193 nm for cyclophosphamide[42] to 195 to 200 nm for cyclophosphamide and ifosfamide (Bristol Myers), to 207 nm for ifosfamide;[30] from to 214 nm to 254 nm, 260 nm, and 266 nm (after methanol extraction) for 5-fluorouracil;[44] to 216-nm for doxorubicin;[34] and 230 nm for paclitaxel.[43,45] These are only a few of the different UV wavelength settings found in the literature search. Subsequently, a UV spectrum analysis using a Beckman DU-600 analyzer (spectrophotometer) was conducted on stock standards of each agent (i.e., in methanol and potassium phosphate buffered water). Because of the limited absorption of UV radiation by CP and IF above 210 nm, it was decided to use 195 nm for the optimum wavelength, where all five agents of interest indicated strong absorption.

HPLC Column Selection

Review of the literature found a variety of separation columns used in the analysis of antineoplastics. From among those, the Waters Symmetry C8, 3.5 μm, 4.6 mm $\times$ 150 mm ID, column (Waters Part No. WAT200630) was chosen for use initially. However, this column was prone to fouling and also caused significant backpressure in the HPLC, even when using a 5.0 μm Symmetry guard column ahead of the column. After the initial evaluation of analytical range, this column was replaced with a Waters Symmetry C18, 5.0 μm, 4.6 mm $\times$ 150 mm ID (Waters Part No. WAT045905). Both columns demonstrated acceptable separation of the components, but the C18 column with guard column was selected for all subsequent analyses on the HPLC because it was much less prone to fouling and did not cause exceptional backpressure.

Mobile Phase

After reviewing mobile phase blends used in other HPLC analytical methods, it was decided to use an acetonitrile:potassium phosphate buffer. A 10-mM potassium phosphate buffer was prepared by combining 1.1936 g of potassium phosphate, monobasic (KH_2PO_4) and 0.2143 g potassium phosphate, dibasic (K_2HPO_4) with 1.0 L of Milli-Q water to form a pH 6.0 buffer. After testing, it was decided the optimum blend for assuring good separation and for maintaining minimal time for the agents of interest was a 22.75 percent acetonitrile: 77.25 percent buffered water combination. All mobile phase blends were filtered through 0.2 μm filters prior to introduction into the HPLC system. The mobile phase usually used for analysis of taxol contains



Min.	Agent	Min.	Agent
2.49	Solvent Front	9.70	Cyclophosphamide
2.89	5-Fluorouracil	17.50	Doxorubicin HCl
8.64	Ifosfamide	39.47	Paclitaxel

FIGURE 1

Typical HPLC chromatogram for all five agents on a single run at AUFS of 0.05 (fluorouracil at 5 μg/mL, ifosfamide and cyclophosphamide at 10 μg/mL, doxorubicin at 20 μg/mL, and paclitaxel at 3 μg/mL).

TABLE II
Information on agents used to prepare standards

Brand name	Composition	Lot no.	Manufacturer
Cytoxan (for injection)	Cyclophosphamide (20 mg/mL)	Lot 9G22823	Meade-Johnson (Bristol Myers Squibb)
Ifex (for injection)	Ifosphamide (50 mg/mL)	Lot KCS99	Meade-Johnson (Bristol Myers Squibb)
Doxorubicin HCl	Doxorubicin HCl (2 mg/mL)	Lot 123200A Lot 93592	Novaplus Bedford Labs
Adricil (for injection)	Fluorouracil (50 mg/mL)	Lot FFA221	Pharmacia & Upjohn
Paclitaxel (99.99%)	Paclitaxel	Not available	Sigma

methanol. K.C. Chan et al. used methanol–acetonitrile–ammonium acetate ratio in a 20:25:55 mix.[35]

Pump Flow Rate

Pump flow rate was also initially tested at 1.0 mL/min and was used during the determination of analytical range for each agent of interest. However, because of the reduction in backpressure when the C18 column was installed, the optimum flow rate was changed to 1.2 mL/min. An isocratic flow was used for 20 minutes, followed by a 30-minute gradient phase. During the gradient phase, the acetonitrile component of the mobile phase was increased from 22.75 percent to 70 percent acetonitrile. This was required for the elution of paclitaxel (taxol).[35,45] The following five minutes was used to reverse the gradient phase back to its original concentration of 22.75 percent acetonitrile, followed by 5 min of operation at the 22.75 percent isocratic phase to assure return to the a stable original baseline (60 min. total run time).

Standard Preparation

Stock standards were prepared in essentially the same fashion for each of the agents of interest. Those that were received in vials in dry form were first reconstituted in accordance with the manufacturer's recommendations. This was usually the addition of a specific quantity of distilled water. To minimize exposures during this reconstitution step, the vial septum was equipped with an air filter to allow for discharge of air as the vial was charged with distilled water.

All agents, those reconstituted to liquid form and those received in liquid form, were first blended with a 50:50 methanol: Milli-Q water blend. For example, cyclophosphamide was reconstituted to 20 mg/mL. An aliquot of 1.0 mL was withdrawn by syringe and placed in a plastic vial (15-mL polypropylene, disposable centrifuge tubes with screw-on cap, order number 430790), followed by an aliquot of 9 mL of the methanol: water blend. This would form a 2.0-mg/mL stock standard. Subsequent cyclophosphamide standards were formed by dilutions of this standard with Milli-Q equivalent water. For example, a 1.0-mL aliquot of the 2.0-mg/mL stock standard and 9.0-mL Milli-Q equivalent water provided a 200-μg/mL standard.

Commercial products contained the pure agents (99.99% purity) and other agents with the exception of taxol. Paclitaxel was tested in pure form due to the presence of other taxanes in the taxol product causing difficulty in identifying the peak of interest. The agents and respective products specifically evaluated are shown in Table II. From these products, stock standards were made that were further diluted to arrive at calibration standards of varying concentrations for each of the five agents being studied. Quality assurance was maintained by using all products prior to their manufacturer's expiration date.

For this portion of the study, determining both the acceptable analytical detection range and validating the method for simultaneous analysis of all five agents, the typical concentrations prepared were 1.0 μg/mL, 2.0 μg/mL, 5.0 μg/mL, and 10 μg/mL. Paclitaxel was validated at 2.0 μg/mL, 5.0 μg/mL, 10 μg/mL, and 20 μg/mL because of difficulty in avoiding precipitating out of the paclitaxel, possibly due to a reduction in methanol present, when further dilutions were attempted.

RESULTS

The typical peak separation and response obtained from using this analytical method on the newer HPLC system is shown in Figure 1 with an analysis using an AUFS of 0.05. It should be pointed out that an analysis conducted on the MAPS 100 with all agents except paclitaxel being at 10 μg/mL, 5-fluorouracil appeared at 3.45 min, ifosfamide at 9.46 min, cyclophosphamide at 10.62 min, and doxorubicin HCl at 15.61 min. The peak for paclitaxel at 5.0 μg/mL appeared at 38.60 min, or about the same time as the newer HPLC system after the initiation of the gradient phase to elute the paclitaxel.

Tables III–VII provide information used for developing the calibration curves for the various contaminants at the respective

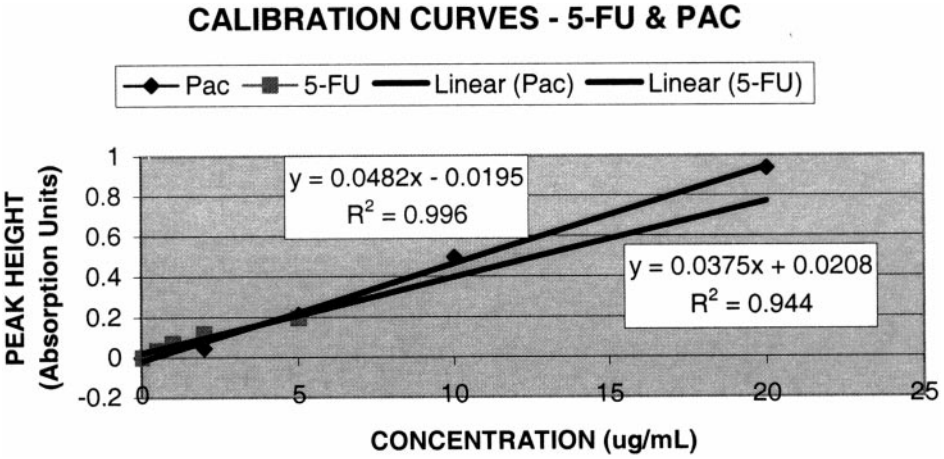


FIGURE 2
Standard curve for 5-fluorouracil based on average peak height at each concentration to 5.0 μg/mL and paclitaxel to 20 μg/mL.

concentrations detected. The following calibration curves (Figures 2, 3, 4, and 5) indicate the linear slope and *y*-intercept for average recoveries observed. The peak heights indicated for 5-fluorouracil, cyclophosphamide, ifosfamide, and doxorubicin HCl are all in computer units (CUs), which are in direct relation to absorption units. All peak heights were obtained by actual measurement of the peak height from the baseline. This was done instead of using the computer units for peak height from the HPLC analytical program because of the difference in recognizing the baseline from analysis to analysis. However, the analyses for paclitaxel did rely on the computer units for peak height due to a problem with the baseline disappearing from observation during the gradient portion in some analyses. Thus, the resulting peaks could not be manually measured and computer determination of peak height was used.

Additionally, the standard curve determined from the average results for each agent at the various test concentrations was fitted with a trend line and also statistically analyzed to determine linearity. Analyses were conducted to develop a "standard curve" for each of the antineoplastics of interest and determine the linearity for each. Except for paclitaxel, results for each antineoplastic studied are discussed in the time order they appear in on the HPLC chromatogram.

Results from analyses conducted on 5-fluorouracil for concentrations from 0.5 to 5.0 μg/mL, which appear on the chromatogram at approximately 3 minutes, found the calibration curve to generally be linear, with an R^2 of 0.9439 (see Table III and Figure 2). It should be noted that an R^2 is the variance of *y* that can be explained by the variable *x*. Thus if $R^2 = 1.00$, then all variations in *y* can be explained by the variation in *x*, and all data points fall on the regression line.[46] However, at the concentration for 10.0 μg/mL, the HPLC detector response was nearly the same as the response for 5.0 μg/mL, indicating the linear portion of the line had been exceeded (Table III and Figure 3). This would indicate an upper limit for detection of 5-fluorouracil of approximately 5.0 μg/mL.

Paclitaxel was tested at concentrations ranging from 2.0 to 20.0 μg/mL and was found to appear on the gradient portion of

TABLE III
Analytical results for 5-fluorouracil to 10.0 μg/mL

	Concentration (μg/mL)				
Fluorouracil	0.5	1.0	2.0	5.0	10.0
Average peak height (AUs)	0.0352	0.0713	0.1194	0.1965	0.1955
Number of samples (n)	5	6	6	5	5
S.D. (AUs)	0.0010	0.0015	0.0002	0.0024	0.0032
C.V. (%)	2.98	2.12	0.19	1.23	1.97
Slope	0.019				
y-intercept	0.046				

TABLE IV
Analytical results for paclitaxel to 20.0 μg/mL

	Concentration (μg/mL)			
Paclitaxel	2.0	5.0	10.0	20.0
Average peak height (AUs)	0.0462	0.2126	0.4929	0.9354
Number of samples (n)	4	6	4	6
S.D. (AUs)	0.003	0.025	0.097	0.033
C.V. (%)	5.8	11.7	19.7	3.5
Slope	0.05			
y-intercept	0.71			

TABLE V
Analytical results for ifosfamide to 10.0 μg/mL

	Concentration (μg/mL)				
Ifosfamide	0.5	1.0	2.0	5.0	10.0
Average peak height (AUs)	0.0013	0.0022	0.0031	0.0093	0.0156
Number of samples (n)	6	6	6	5	4
S.D. (AUs)	0.00004	0.00005	0.00006	0.00025	0.00066
C.V. (%)	3.31	2.31	2.06	2.69	4.23
Slope	0.0016				
y-intercept	0.0004				

the analysis at approximately 39 minutes. Although the R^2 was found to indicate minimal variance with concentration having an R^2 of 0.996, there was much variation in both the standard deviation (from 4.0 AUs at 2.0 μg/mL to 145.5 AUs at 10.0 μg/mL) and coefficient of variation (from 3.7% to 19.7%) at the concentrations tested (see Table IV and Figure 2).

Results from analyses conducted on ifosfamide and cyclophosphamide for concentrations ranging from 0.5 to 10.0 μg/mL, which appear on the chromatogram at approximately 8 and 9 min, respectively, were both found to have minimal variance with R^2 values of 0.9906 and 0.9703. Accuracy and precision were also very acceptable for ifosfamide and cyclophosphamide with most standard deviations less than 1.0 AU and coefficients of variation less than 5.0 percent (see Tables V and VI and Figures 4 and 5).

Doxorubicin HCl results for concentrations ranging from 0.5 to 5.0 μg/mL, which appears on the isocratic portion of the analysis at approximately 14 minutes, were found to have minimal variation with an R^2 of 0.9883 (see Table VIII and Figure 4), but this deteriorated slightly with an R^2 of 0.9252 when analysis at 10.0 μg/mL was included (Figure 5). The standard deviation ranged from 0.06 to 2.45 AUs and the coefficient of variation ranged from 2.0 percent to 15.5 percent at the concentrations tested. This was much more variation in both the standard deviation and coefficient of variation at the concentrations tested than with the ifosfamide and cyclophosphamide. The most noticeable concern for doxorubicin was in it being observed to deteriorate in a matter of minutes when in contact with some of the other agents, such as 5-fluorouracil.

STABILITY TESTING

These stability tests were conducted on each agent using standard solutions of 10.0 μg/mL for 5-fluorouracil, ifosfamide, cyclophosphamide, and doxorubicin HCl. A 3.0-μg/mL solution was used for paclitaxel because of its increased response to the UV wavelength setting.

Each agent was initially prepared as a stock standard using a 50:50 blend of methanol-buffered water (6.0 pH) then diluted with Milli-Q equivalent water to the appropriate concentration for test purposes. These solutions were maintained at room (ambient) temperatures and analyzed on the days indicated for each. The results for each antineoplastic studied are discussed in the order it appears on the HPLC chromatogram. Analyses were attempted at additional times to those shown in the tables below, but because of difficulties with the HPLC, either with pressure drops in the mobile phase or detector problems, these results were not usable.

TABLE VI
Analytical results for cyclophosphamide to 10.0 μg/mL

	Concentration (μg/mL)				
Cyclophosphamide	0.5	1.0	2.0	5.0	10.0
Average peak height (AUs)	0.0013	0.0018	0.0029	0.0092	0.0135
Number of samples (n)	6	6	6	6	4
S.D. (AUs)	0.00005	0.00006	0.00004	0.0005	0.0012
C.V. (%)	3.62	3.15	1.26	5.76	9.10
Slope	0.0014				
y-intercept	0.0005				

TABLE VII
Analytical results for doxorubicin HCl to 10.0 μg/mL

Doxorubicin HCl	Concentration (μg/mL)				
	0.5	1.0	2.0	5.0	10.0
Average peak height (AUs)	0.0029	0.0046	0.0086	0.0286	0.0356
Number of samples (n)	6	6	6	6	4
S.D. (AUs)	0.0001	0.001	0.001	0.002	0.002
C.V. (%)	2.04	18.83	6.72	6.96	6.90
Slope	0.0036				
y-intercept	0.0026				

As indicated in the Results section above, 5-fluorouracil is the first agent to appear on the chromatogram after the solvent peak (about 3 minutes). Analyses were conducted on the day prepared (day 0), and days 6, 8, 17, 27, and 31. These results indicated an approximate deterioration of 20 percent in the first week, and approximately 41 percent over a 31-day period (Figure 6). Paclitaxel was tested at day 0 and days 6 and 7. Only a small fraction of deterioration, less than 2 percent, was observed (Figure 6).

Ifosfamide and cyclophosphamide were tested at 0, 1, 2, and 14 days. The recovery at day 2 indicates an average deterioration of 13 percent after 2 days and a 32 percent deterioration after 14 days for ifosfamide, and no detectable deterioration at day 2 but a 33 percent deterioration after 14 days for cyclophosphamide (Figure 7). Doxorubicin HCl was tested at 0, 6, and 17 days. The average deterioration at day 6 was approximately 45 percent, and on day 17 was approximately 75 percent (Figure 7).

DISCUSSION

Of primary importance for this study was the determination of an acceptable UV wavelength for the simultaneous detection of the five agents of interest. Other aspects, such as mobile phase composition and peak separation were also important, but it was most important to determine if all five agents could be seen at the same wavelength. Settings of the UV wavelength for detection of cyclophosphamide in various reported studies have ranged from 193 nm to 270 nm.[35,41,42,47] An analysis by a spectrophotometer provided the information needed to determine an optimum UV wavelength (195 nm) for this analytical method.

For information purposes, a wavelength of 254 nm was tested one time during this study because of it appearing in some of the literature for general analyses of antineoplastics, but, as expected, it was not found to be a usable wavelength for this method.

Results from the tests on 5-fluorouracil indicated that it could be detected separate from the solvent peak on the chromatogram at concentrations of approximately 1.0 μg/mL or less. For higher concentrations, the analysis had to be reconducted with a higher AUFS (sensitivity) setting to separate the 5-fluorouracil from the solvent peak for both identification and quantification.

Cyclophosphamide, the agent of most interest in this study because of its frequency of use and its being recognized as a human carcinogen, and ifosfamide, a similar type of agent, were both found to be very accurately measured to levels of 0.5 μg/mL. Based on the increased sensitivity observed when the newer HPLC was used in the stability testing, it is expected that the lower minimum detection limit is likely to be 0.1 to 0.2 μg/mL. This would make this method competitive with use of the HPLC-Mass Spec systems for acceptable low-level detection capabilities, but without the derivatization step and higher equipment and time costs associated with the Mass Spec method.

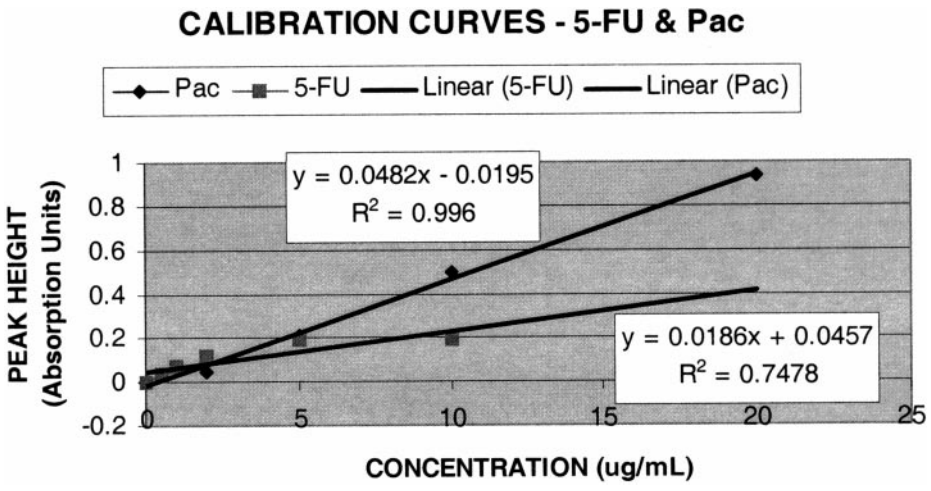


FIGURE 3
Standard curve for 5-fluorouracil based on average peak height at each concentration up to 10.0 μg/mL and paclitaxel to 20 μg/mL.

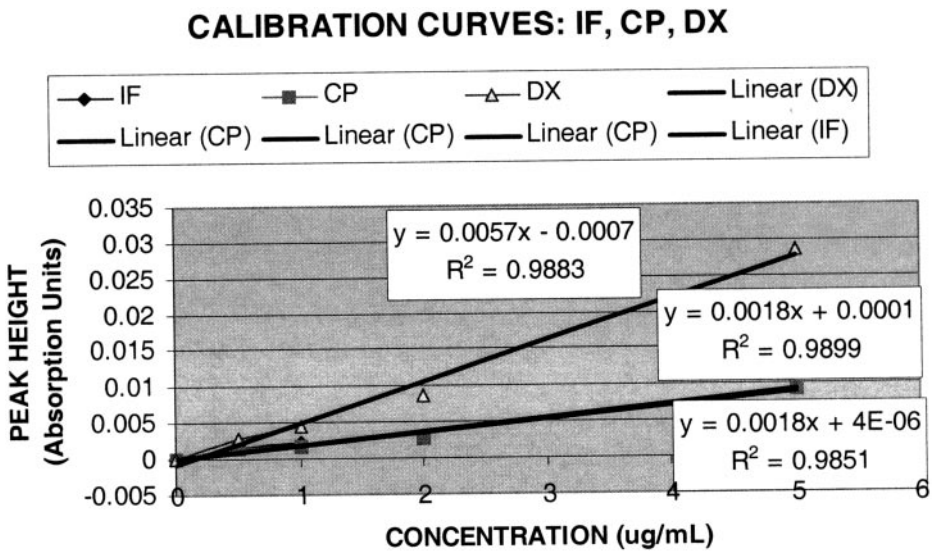


FIGURE 4
Standard curves for ifosfamide, cyclophosphamide, and doxorubicin HCl based on average peak height at each concentration up to 5.0 μg/mL.

Both standard curve and stability test results for doxorubicin HCl appeared to be acceptable, but this was for the pure agent. It was noted that when doxorubicin HCl is blended with some other agents or comes in contact with surfaces containing various organic chemicals, the deterioration can be very rapid with as much as a 30 percent deterioration in concentration in one day.

Some difficulty was observed in making paclitaxel standards. It was found that paclitaxel is less soluble in solvents other than methanol and that a precipitate would form or the paclitaxel would not completely dissolve in some blends of methanol, acetonitrile, and water. This caused some difficulty for both the development of a standard curve and a stability analysis. Although both were accomplished with this method, the results did indicate a relatively wide fluctuation in results for analysis of the agent at the same concentration (high standard deviation and coefficient of variation).

CONCLUSIONS

Overall, results of this study found the analytical method described above to be acceptable for the detection and identification of all five agents of interest in a single analysis. The lower limit of detection, based on results from using the MAPS 100

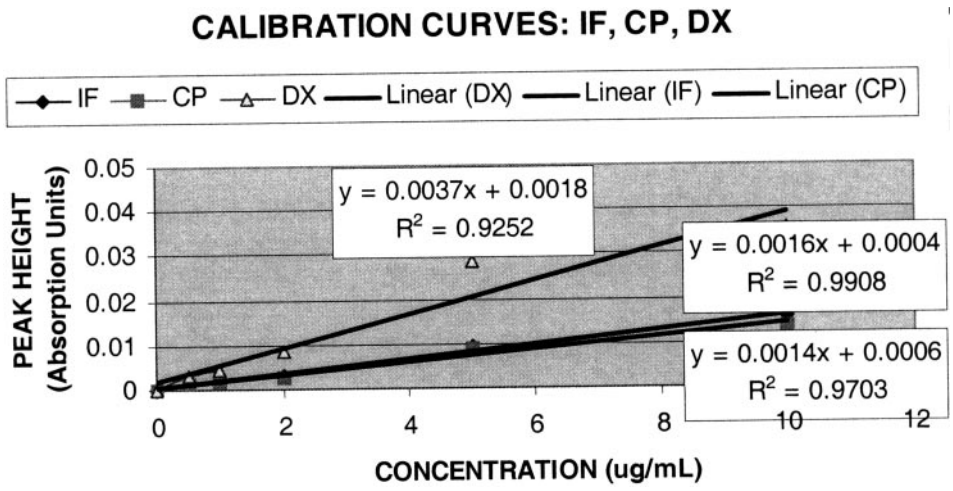


FIGURE 5
Standard curves for ifosfamide, cyclophosphamide, and doxorubicin HCl based on average peak height at each concentration up to 10.0 μg/mL.

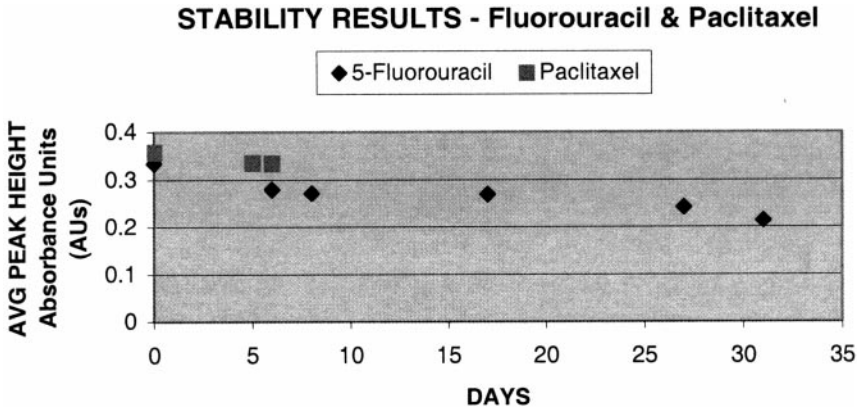


FIGURE 6

Stability test results for 5-fluorouracil based on testing at a 10.0 μg/mL concentration over a period of 31 days, and paclitaxel based on testing at 3.0 μg/ml concentration over a period of 7 days.

HPLC, is 0.5 μg/mL for four of the agents: 5-fluorouracil, ifosfamide, cyclophosphamide, and doxorubicin HCl. Paclitaxel's lower limit of detection was determined to be 2.0 μg/mL on this unit. Quantification of these agents is also acceptable for these agents, except that it may be necessary to reconduct the analysis at a higher AUFS for the purposes of quantifying 5-fluorouracil.

The stability results did indicate varying degrees of deterioration when stored at ambient temperatures. This information supports storing standards at -20°C and that both standards and samples should be assayed as soon as possible after the samples are collected to avoid error due to loss of potency. Ideally, fresh stock standards should be prepared for each day of analyzing for these agents.

Because this method has been found to be an effective tool for the simultaneous analysis of five different antineoplastics, it provides the capability to conduct a more comprehensive evaluation of facilities handling antineoplastic agents. For example, analysis can be conducted on samples collected from various surfaces commonly found in hospitals and pharmacies, or on solid sorbents used to collect air contaminant samples. Data from such samples can provide valuable information on exposure potential to individuals working in such facilities, both the potential for exposure via skin contact with contaminated surfaces or exposure to contaminated air within their work area.

Additionally, this method provides the ability to conduct accurate risk analysis of individuals working in areas where these agents may be handled. This is very important because of the need for more accurate information to associate exposure information to actual dose concentrations (e.g., uptake in the body) of these agents detected in the urine of oncology pharmacists and nurses.[37,48,49]

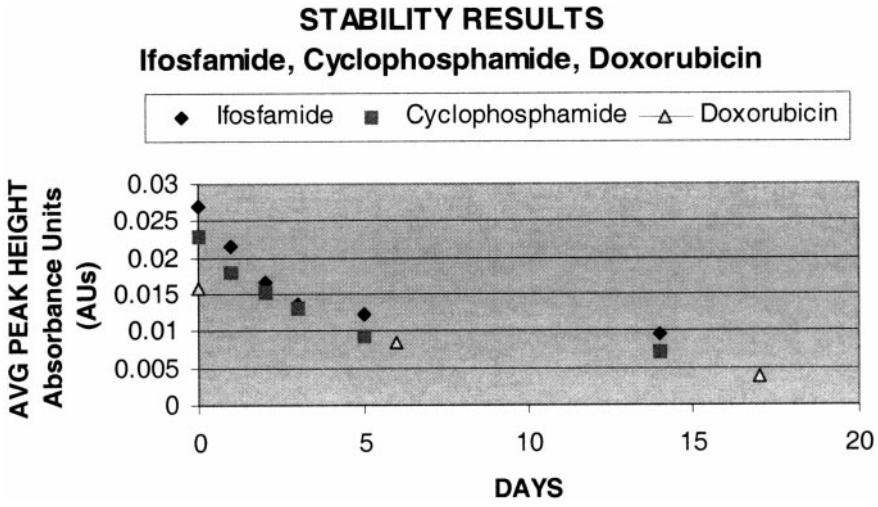


FIGURE 7

Stability test results based on testing of 10.0 μg/mL solutions of ifosfamide and cyclophosphamide over a period of 14 days, and 5.0 μg/mL solution of doxorubicin over 17 days.

RECOMMENDATIONS

This analytical method can be used for the analysis of the desorbing liquid extracted from either air or surface monitoring samples. It is recommended that surface and/or air samples be extracted with a mixture of 10 percent acetonitrile, 25 percent methanol, and 65 percent Milli-Q or equivalent water (all solvents HPLC grade). This mixture provides better extraction of the agents of interest for this analytical method. For air monitoring samples or surface samples that do not require analysis for paclitaxel, the 20-minute isocratic method of analysis is acceptable.

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