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Journal of Chromatography B, 676 (1996) 165–168

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Short communication

Direct injection isocratic high-performance liquid chromatographic analysis of mitomycin C in plasma

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Received 13 July 1995; revised 1 September 1995; accepted 19 September 1995

Abstract

A direct injection high-performance liquid chromatography method is described for the determination of mitomycin C (MMC) in human plasma. The stationary phase consisted of hydrophilic and hydrophobic functional groups covalently bound to silicone-coated silica beads (CAPCELL PAK MF Ph-1, 150×4.6 mm I.D., 5 μm). A mobile phase using 100% water gave a better separation of MMC from endogenous interferences as compared to a mobile phase with 12.5% acetonitrile and 2.5 m*M* phosphate buffer (pH 6.9). Using water as the eluent (1 ml/min) and UV detection at 365 nm, MMC was found to elute at 5.0 min with a peak width of 0.3 min, whereas endogenous interferences eluted before 3 min. Total assay time per sample was 6 min. Internal standard was not required because the recovery of MMC was nearly complete, about 90% from 20 to 5000 ng/ml. The standard curve was linear from 20 to 5000 ng/ml in plasma, and the intra- and inter-day variation was between 3 to 6%. The lower detection limit was 5 ng/ml with a 25 μl sample, which represents a two- to four-fold improvement over the 10 ng/ml detection limit by previous methods using liquid–liquid extraction and comparable sample size. The simplicity of this method, i.e., no sample extraction, no internal standard, 100% aqueous mobile phase, isocratic elution and short analysis time (6 min/sample), makes it suitable for large scale routine sample analysis, whereas its small sample volume requirement and high sensitivity are useful for pharmacokinetic studies in small animals where limited sample is available.

Keywords: Mitomycin C

1. Introduction

Mitomycin C (MMC) is an antitumor antibiotic which has shown clinical activity in a number of cancers, including stomach, breast, pancreas, colon, and bladder cancer [1]. Previous analysis of MMC in biological samples required sample preparation, such as liquid– liquid extraction [2,3], solid-phase extraction or protein precipitation [3], prior to analysis by reversed-phase high-performance liquid chromatography (HPLC). Elimination of the sample clean-up procedures will facilitate the analysis.

HPLC columns that permit direct injection of plasma without extensive sample clean- up often employ so-called restricted access materials such as internal-surface reversed phases, shielded hydrophobic phases, and semi-permeable surfaces [4]. These packing materials are unique in that matrix com-

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0378-4347/96/$15.00
SSDI 0378-4347(95)00406-8

ponents such as proteins are allowed to interact only with hydrophilic, non-adsorptive layers on the outer packing surfaces, whereas small molecules such as drugs and their metabolites are selectively retained and are allowed to penetrate and gain full access to the internal reversed-phase coating materials. The unique feature of the commercially available CAPCELL PAK MF Ph-1 column is its silicone-coated silica beads, which minimize the undesirable secondary interactions with the silica surface [5].

This report describes a method where MMC in plasma can be directly analyzed by isocratic HPLC without sample preparation.

2. Experimental

2.1. Materials and reagents

MMC was a gift from Bristol-Myers (Wallingford, CT, USA). All HPLC solvents were of the HPLC grade and were filtered and degassed before use.

2.2. Solutions and standards

Stock solutions of MMC (0.5 mg/ml) were prepared in distilled water and stored at −70°C. Standard solutions of MMC were prepared by dilution of the stock solution with water to give final MMC concentrations between 5 to 5000 ng/ml. Standard solutions of MMC in human plasma were prepared by spiking plasma with appropriate volumes of MMC stock solution to give final MMC plasma concentrations between 20 to 5000 ng/ml.

2.3. Apparatus and chromatographic conditions

The liquid chromatograph consisted of an Hitachi Model L-6200A Intelligent pump (Hitachi Instruments, Naperville, IL, USA), a Waters 717 autosampler (Waters Chromatography Division, Milford, MA, USA) with a 200-μl injection loop, an on-line precolumn filter (0.45 μm, Upchurch Scientific, Oak Harbor, WA, USA), an HP 1050 variable wavelength UV detector, and an HP 1040A diode-array detector (Hewlett-Packard, Avondale, PA, USA) connected in sequence after the variable wavelength UV detector. The HPLC system was controlled by the HPLC Chemstation data system (Hewlett-Packard).

The HPLC column was CAPCELL PAK MF Ph-1 (150×4.6 mm I.D., 5 μm, Shiseido, Tokyo, Japan), a new type of internal surface reversed-phase (ISRP) column that contained a mixed-functional (MF) phase coating on silicone polymer coated silica beads [5]. The mixed-functional phase consisted of an outer surface of hydrophilic polyoxylene and an inner surface of hydrophobic phenyl groups. Plasma samples were centrifuged at 2000 g for 15 min, and 25 μl of the supernatant was injected onto the HPLC system. The eluent was monitored at 365 nm with a variable wavelength UV detector, and with a diode-array detector to monitor the UV spectrum on-line.

2.4. Assay validation and recovery

Standard curves of MMC in human plasma were constructed for a concentration range of 20 to 5000 ng/ml. The standard curve and the control samples were prepared separately. Intra-day and inter-day variations were determined at three MMC concentrations, i.e., 50, 500, and 1000 ng/ml over a period of 7 days. Recovery was determined as the ratio of the UV absorbing peak height obtained from MMC-containing plasma samples to the peak height from MMC-containing aqueous standard solutions.

3. Results and discussion

Using the CAPCELL PAK MF Ph-1 column, we found that a mobile phase consisting of 12.5% acetonitrile and 2.5 mM phosphate buffer (pH 6.9), which was used successfully for separation of MMC on a C_{18} reversed-phase HPLC column [2], gave a poor separation of MMC from endogenous interferences (data not shown). On the other hand, pure water gave an excellent separation.

Fig. 1 shows the HPLC chromatograms of a blank plasma sample and a plasma sample containing 50 ng/ml MMC. The retention time of MMC was 5.0 min, whereas the endogenous components eluted before 3 min. The capacity factor of MMC peak was 5.25, with a inter-day coefficient of variation of 1.2% as determined over a period of 7 days (n=16). The intra-day coefficient of variation was <1%. To

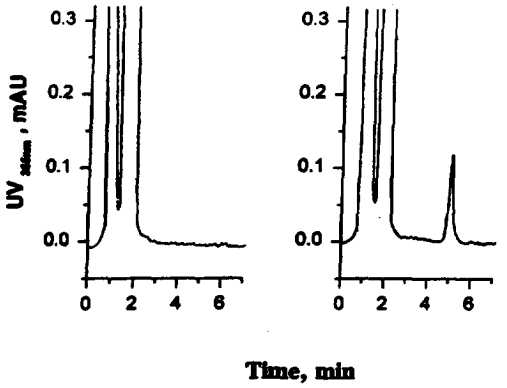

Fig. 1. Representative chromatograms of MMC in human plasma. (A) Blank plasma; (B) plasma containing 50 ng/ml MMC.

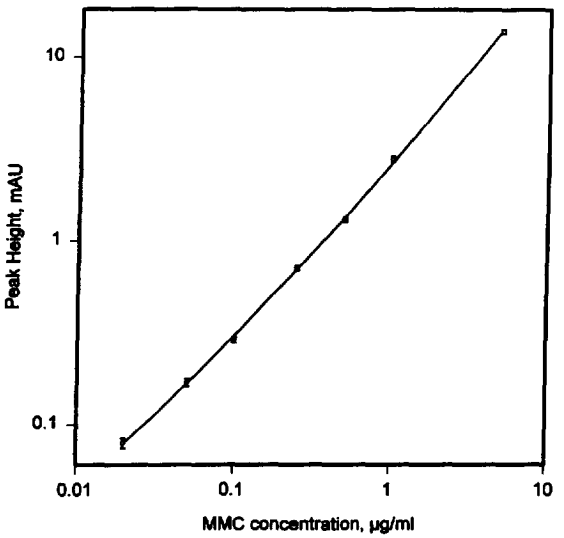

Fig. 2. Standard curve of MMC in plasma (mean ± S.D., $n=3$). Note that the standard deviation for some data points was smaller than the symbols.

determine if the assay is specific for MMC, two procedures were used. The first was to compare the UV spectrum of the eluting peak obtained by on-the-fly scanning using the diode array detector with the spectrum of pure MMC. The data showed identical UV spectra, indicating high purity of MMC in the eluting peak. The second method was to determine if there were interferences hidden in the elution window of MMC (4.5 to 6.0 ml). The baseline noise level of a blank plasma sample was determined objectively by the computerized data system as six times the standard deviation of the baseline signal obtained and was 0.002 mAU. The baseline noise levels for a blank plasma sample and a pure water sample were identical, thus confirming that no endogenous interferences appeared in the elution window of MMC. It is noted that the efficiency of the CAPCELL PAK MF Ph-1 column for MMC was lower than conventional reversed-phase C_{18} columns. However, its unique application for direct plasma injection warrants its application in routine bioanalysis.

The standard curve was linear over the range of 20 to 5000 ng/ml of human plasma (Fig. 2). The regression equation for this line was: peak height (mAU) $=2.735\times$MMC concentration (μg/ml) $+0.0244$, with a coefficient of determination (r^2) of 0.999. The concentrations obtained using the standard curve and the UV detection system for the control samples of 50, 500, and 1000 ng/ml, were 52.1, 488.4, and 967.5 ng/ml, respectively. The coefficients of variation at the three concentrations were 5.2, 3.1, and 2.3% for samples analyzed on the same day (intra-day), and were 7.8, 3.3, and 5.9%, for samples analyzed on different days (inter-day). These data indicate a high precision and accuracy by the described assay procedures.

Analysis of MMC using standard HPLC methodologies is complicated by its polar nature, adsorption to glass surfaces, and relative aqueous instability. The polar nature of MMC contributes to a low recovery by liquid–liquid extraction. MMC adsorption to glass surfaces, in particular during evaporation, is also recognized as a factor reducing recovery [2]. Recovery of the adsorbed MMC is usually unsuccessful. MMC degradation in aqueous media is accelerated by low and high pH, buffer ions, and elevated temperatures [3]. For example, MMC degrades with a half-life of less than 2 h at 37°C and a pH of 5.0 in human urine [6]. These factors result in a low and variable recovery. A recent study reported a liquid–liquid extraction recovery ranging from 58 to 91% from tumor homogenate [7]. By tediously avoiding evaporation of the MMC-containing organic solvent to complete dryness while maintaining neutral pH and temperature below 25°C, we obtained a recovery of 93.1±4.7% after extraction with ethyl acetate from plasma and urine [2]. The present study showed that direct injection of MMC-containing

plasma with no sample preparations gave a consistent and high recovery (90.6±3.5%, $n=7$), at a concentration range of 20–5000 ng/ml. The consistent and high recovery obtained with the direct injection method allows eliminating the use of an internal standard, thus simplifying the assay procedure.

The lower detection limit of this method was 5 ng/ml for a 25 μl plasma sample at a signal-to-noise level of 3. This represents a two- to four-fold improvement over the 10 ng/ml detection limit by previous methods using liquid–liquid extraction and comparable sample size [3]. It is noted that other methods, using larger sample volume (e.g., 1 ml) and liquid–liquid extraction, can give a higher sensitivity of about 1 ng/ml [3]. The present method is a useful alternative when the higher sensitivity is not required. In our laboratory, this assay is used to measure the MMC concentration in urine and bladder tissues in animals and human patients.

The small sample volume (25 μl) used in this method is particularly suited for pre-clinical studies with small animal species (i.e., rodents) where sample volume is limited. The simplicity of this method, i.e., no sample extraction, no internal standard addition, 100% aqueous mobile phase, isocratic elution, and short assay time (6 min/sample) makes it ideal for large scale routine sample analysis.

4. Conclusion

A direct plasma injection HPLC method using the CAPCELL column was developed for the analysis of MMC in plasma samples. This method does not require additional sample preparation steps (e.g., internal standard, extraction), requires only a small sample volume (25 μl), shows a $< 8\%$ inter-day and intra-day variability, and has a sensitivity of 5 ng/ml.

Acknowledgments

The authors thank Dr. M. Guillaume Wientjes for his valuable suggestions. Supported in part by a research grant R37CA49816 and a Research Career Development Grant (K04CA01497) to Jessie L.-S. Au from the National Cancer Institute, NIH, DHHS.

References

[1] R.T. Dorr and D.D. Von Hoff, Cancer Chemotherapy Handbook, Appleton and Lange, Norwalk, CT, 2nd ed., 1994, p. 717.
[2] J.T. Dalton, E.R. Geuns and J.L.-S. Au, J. Chromatogr., 495 (1989) 330.
[3] J.H. Beijnen, A. Bult and W.J.M. Underberg, Mitomycin C, in Analytical Profiles of Drug Substances, Vol. 16, Academic Press, 1987, p. 361.
[4] P. Campíns-Falcó, R. Herráez-Hernández and A. Sevillano-Cabeza, J. Chromatogr., 619 (1993) 177.
[5] T. Kanda, H. Kutsuna, Y. Ohtsu and M. Yamaguchi, J. Chromatogr. A, 672 (1994) 51.
[6] J.T. Dalton, M.G. Wientjes, R.A. Badalament, J.R. Drago, and J.L.-S. Au, Cancer Res., 51 (1991) 5144.
[7] J. Cummings, L. Chirrey, N. Willmott, G.W. Halbert and J.F. Smyth, J. Chromatogr., 612 (1993) 105.