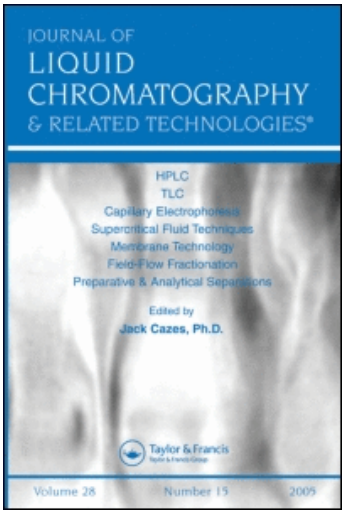


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# Direct Injection Analysis of Mitomycin C in Biological Fluids by Multidemension High Performance Liquid Chromatography with a Micellar Mobile Phase

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# DIRECT INJECTION ANALYSIS OF MITOMYCIN C IN BIOLOGICAL FLUIDS BY MULTIDEMENSION HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH A MICELLAR MOBILE PHASE

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## ABSTRACT

This paper describes the direct injection analysis of the anti-cancer drug mitomycin C in biological fluid by an HPLC column switching technique.The first chromatographic column which provides for sample extraction and clean up employs a micellar mobile phase with SDS as the modifier. The second column, coupled on-line to the first, utilizes reverse-phase conditions for analysis. UV detection is employed at 365nm. Samples from cancer patients and dogs were analyzed. The drug recoveries are 95.9 - 100.3% with a relative standard deviation of 2.76%. A sample analysis is completed within 15 minutes.

## INTRODUCTION

Developments in the technique of HPLC have enabled it to become a very important method for drug studies on the metabolism of drugs. The most straightforward method of sample preparation is liquid-liquid extraction or liquid-solid distribution together with evaporation to recover and concentrate the drug and remove endogenous proteins that can be harmful to the column packings. Such procedures are laborious and time-consuming and the recovery of drug is often low. A more practicable solution to this problem is the use of a simple direct injection technique.

The use of micellar chromatography has been reported to allow such direct injection of biological fluids (serum, plasma and urine).[1-4] Micellar chromatography employs surfactants as mobile phase modifiers[5] to remove protein in untreated biological samples but gives only moderate chromatographic efficiency due to poor mass transfer. Furthermore, the sensitivity of determination is inadequate for many applications.



In recent years column switching combined with the use of micellar clean-up prior to liquid chromatography has been introduced.[6-10] Sodium dodecyl sulphate (SDS)is added to the mobile phase for loading the untreated biological samples onto a precolumn. The proteins are solubilized by the SDS and washed out whereas the analyte is retained. Next, the retained analyte is eluted with the analytical mobile phase and analyzed using conventional RP-HPLC. This method has the advantage of both micellar chromatography (direct biological samples injection, extended column life and good drug recovery) and RP—HPLC (high column efficiency).

Mitomycin C(MMC) has been widely used for cancer treatment in recent years, but its clinical applications are limited owing to inherent severe toxicity. The therapeutic efficacy of mitomycin C is closely related to the drug concentration in blood and tissues, depending on the dosage, route and frequency of administration. Therefore, an appropriate analytical assay that is simple, rapid and reliable is desired.

The determination and quantitation of MMC in biological fluids was mostly performed by means of HPLC methods. The major sample pretreatment procedure for MMC, such as liquid-liquid extraction[11] or offline liquid-solid extraction[12] are in general laborious or very time consuming for large numbers of samples. Other methods,[13-15] which use complicated equipment, have some difficulties in clinical applications.

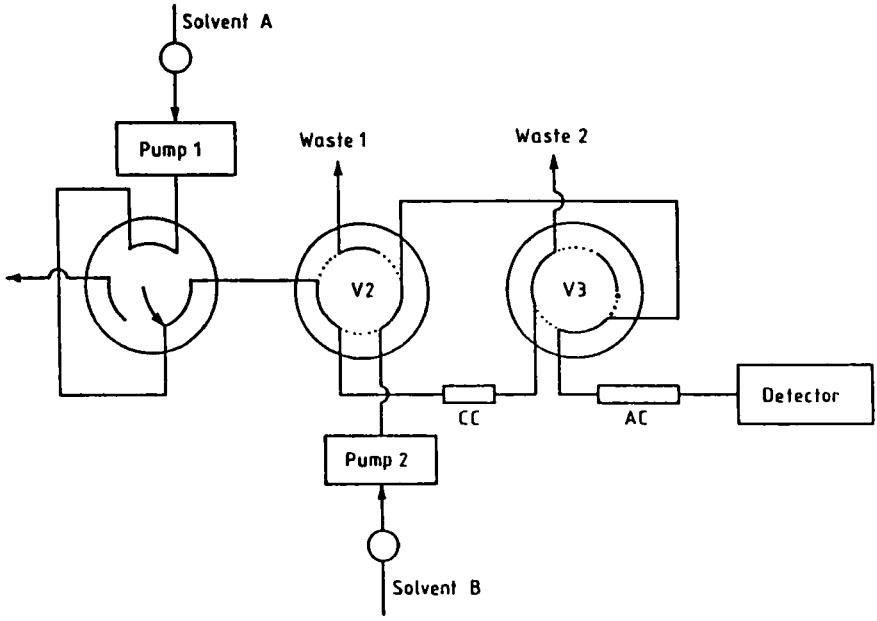


**Figure 1.** Schematic Diagram of Multidimensional HPLC.



In our laboratory, the column switching system is used for the direct injection analysis of MMC in biological fluids. This method is simple, rapid and reliable.

## MATERIALS

### Equipment

A diagram of the experimental set-up is shown in Figure 1. Solvent delivery was provided by two pumps. Pump 1 (Model YSB-2, Shanghai Science Instrument Factory, China) was used for loading the sample onto the clean-up column (CC). Pump-2 (Mode SY-5020, Beijing Analytical Instrument Factory, China) was used to deliver the chromatographic mobile phase. The switching system consisted of a Rheodyne injection valve (Type 7125) with a 10μL sample loop and two K501 injection valves (Shanghai Science Instrument Factory, China). A Mode SPD-I UV/VIS detector (Shimadzu, Kyoto, Japan) was used

for monitoring the effluent from the analytical column (AC). Data were acquired with a C-R2B chromatographic data system (Shimadzu, Kyoto, Japan).

## Column

The two column cartridges used in this study were manufactured by the Beijing Analytical Instrument Factory (Beijing, China). Both the analytical column (150 x 4.6mm i.d., 5μm spherical porous particles) and the clean-up column (50 x 4.6mm i.d., 5μm spherical porous particles) were packed with RP-18 (Shanghai Chemicals Factory, China).

## Chromatographic Conditions

Solvent A: the micellar clean-up eluent, methanol/water (10/90, v/v) containing 20mM SDS (adjusted to pH = 6.8 with 2N HCl).

Solvent B: methanol / water (30/70, v/v), adjusted to pH = 6.8 with 2N HC1.

Flow rate: 1.0mL/min
Detection: UV absorption (365nm)



## Materials and Reagents

The standard MMC was obtained from Kyowa Hakko Kogyo (Tokyo, Japan). Biological samples from cancer patients and dogs were obtained from Gansu Province Tumours Hospital. Sodium dodecyl sulfonate (SDS) was the chemical pure reagent of Xian Chemical Reagent Plant (Xian, China), recystallized twice with alcohol before use. Blood plasma was obtained from Gansu Province Tumers Hospital.

## Assay Procedure

When mobile phase was delivered through the solid lines shown in Figure 1, solvent A (a weak eluent) was carried into CC by pump 1, the dissolved proteins and other weakly retained interfering biological components were rinsed out from CC to waste while MMC was still retained in CC. After eliminating proteins, valves 2 and 3 were switched into the transfer position and the mobile

phase was pumped through the line shown dotted in Figure 1. MMC was thus transferred from CC to AC by solvent B (strong eluent). Then, valves 2 and 3 were rotated back and MMC was eluted and separated by solvent B on AC. Finally, MMC in the biological samples were determined by UV detection.

### Optimization of Mobile Phase and Switch Time

As described above, we know that it was necessary to rinse the cleanup column with a weak solvent to wash out the proteins and other interfering biological components whilst the MMC was retained in CC. We found that when 10% aqueous methanol containing 20mM SDS was used as the weak solvent to flush CC at a flow rate of 1mL/min, the protein and interfering components were eluted within 5 min, but MMC was still in the pre-column.

If 30% aqueous methanol was used, the MMC was eluted within 2 min. Therefore, 10% aqueous methanol containing 20mM and 5 min were chosen as solvent A and switching time 1, respectively. Between 5 min and 9 min the whole of the MMC was delivered into AC. After 9 min, valves 2 and 3 were rotated back. AC was eluted by solvent B while CC was again equilibrated by solvent A. This operating method not only minimizes analytical time, but also avoids prolonged high back-pressure in the chromatographic system.



## RESULTS AND DISCUSSION

### Chromatographic Separation and Calibration Curve

Chromatograms of MMC in biological sample are shown in Figure 2. Blank plasma of patient gave some system peaks (Fig. 2a), but did not interfere with the determination. Figure 2b is the chromatogram of blank plasma of patient with added MMC. Figure 2c and 2d are chromatograms of patient's and dog's plasma, respectively. The results show this method to be suitable for the separation of both synthetic and real samples.

Using the chromatographic condition of Figure 2 the peak height was plotted against the concentration of MMC to give a calibration curve (Fig. 3), with a correlation coefficient of 0.9992 and whose regression is: $Y= 1.17x-0.23$. The linear range of MMC is 1 to 400mg /L.

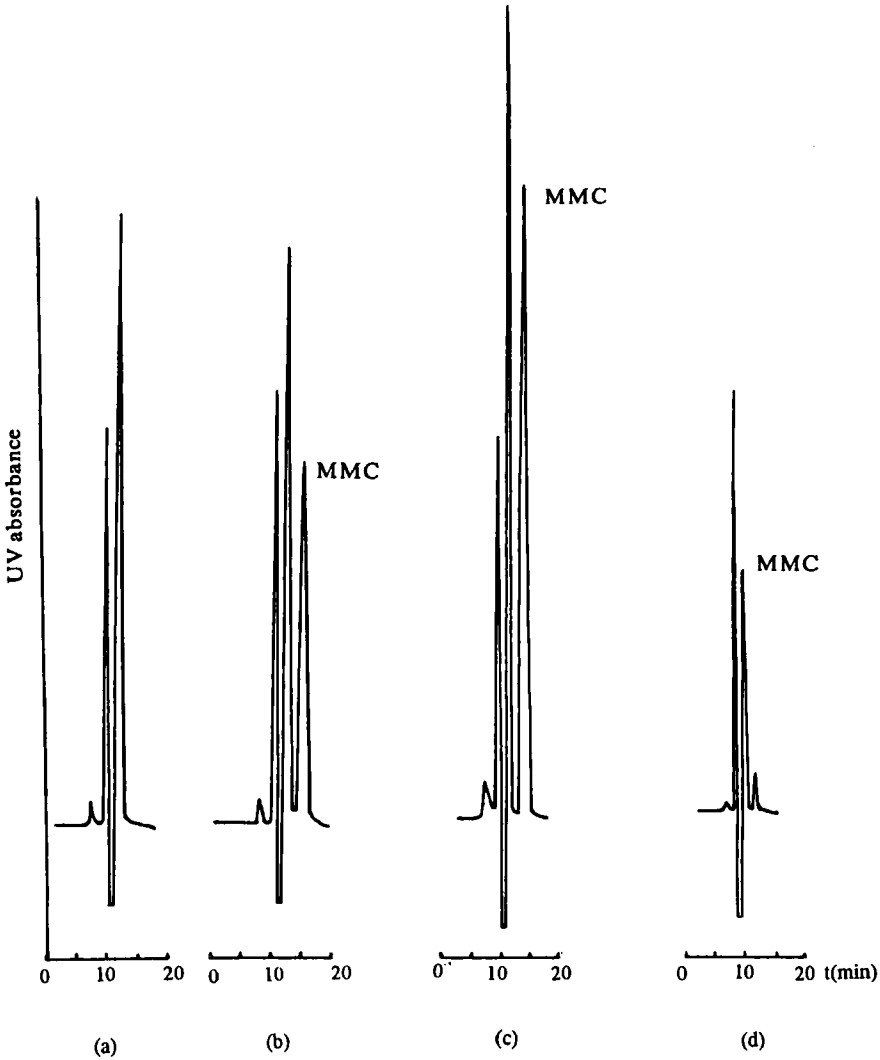


**Figure 2.** Chromatogram of MMC. (a) Blank Plasma; (b) Blank Plasma with Added MMC; (c) Patient's Plasma Sample; (d) Dog's Plasma Sample.

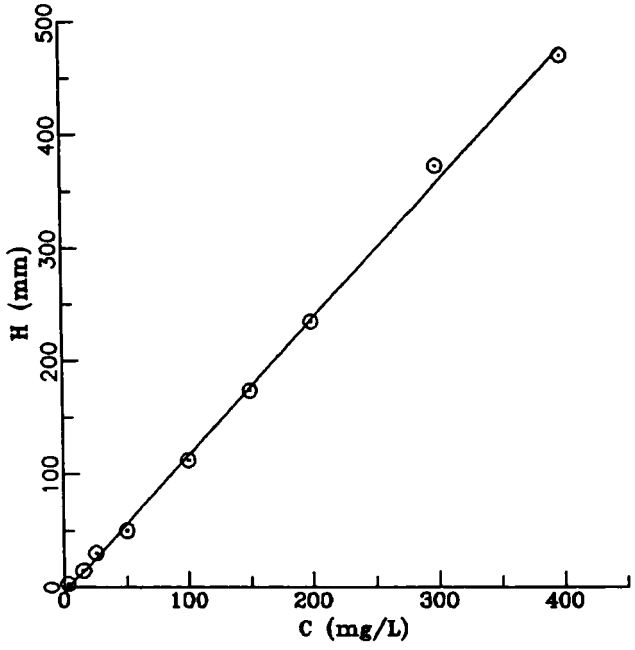


**Figure 3.** Calibration Curve of MMC.



## Recovery and Reproducibility of MMC

Different quantities of MMC was spiked to patients' and dog's plasma samples and the recoveries were calculated according to the following equation:

$$\text{Recovery} = [(S_m\text{-}B) / S \times 100\%]$$

where; S = the peak height given by a standard solution of MMC;B = the peak height of MMC in patients' or dog's plasma; $S_m$ = the total peak height after MMC was added to the patients' or dog's plasma.

The recoveries were 95.9 - 100.3%, as shown in Table 1. The reproducibility was determined by an arbitrary plasma sample. The Relative Standard Deviation (R.S.D.)is 2.76% (n= 5) as shown in Table 2.

**Table 1**

**Recovery of MMC**

| Sample | B | S | $S_m$ | Recovery (%) |
|---|---|---|---|---|
| 1 | 0.0 | 15.4 | 15.9 | 100.3 |
| 2 | 6.7 | 5.75 | 12.3 | 97.8 |
| 3 | 3.05 | 12.17 | 15.2 | 99.8 |
| 4 | 6.45 | 18.45 | 24.65 | 95.9 |

1. The blank plasma of patient
2. The human plasma of locaiton
3. The human plasma of all body
4. The plasma of dog

B, S, $S_m$ : Concentrations in terms of peak height (mm)



**Table 2**

**The Reproducibility of MMC**

| Peak Height | Relative Error (%) | R. S. D. (%) |
|---|---|---|
| 113.4 | 2.84 | |
| 112.0 | 1.44 | |
| 112.0 | 1.44 | 2.76 |
| 115.6 | 4.96 | |
| 111.0 | 0.56 | |

**Determination of MMC in Patients's / Dog's Plasma**

The direct local administration on tumour will give higher drug concentration and lower toxicity than those of general administration. Patients' and dog's plasma samples after administration were analyzed in our study. Patients and dogs were given MMC at a dosage of 10mg and 6mg, respectively. Figure 4 and Figure 5 show the data of the analysis. We can observe higher MMC concentration of local administration than that of general administration at same dosage.

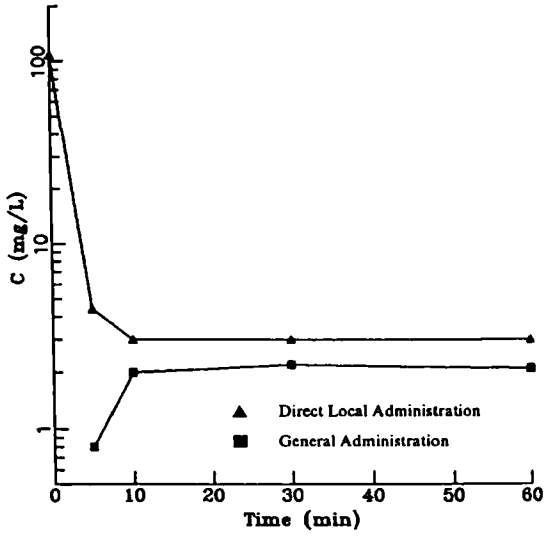


**Figure 4.** Elimination Curve of MMC in Patient's Plasma.

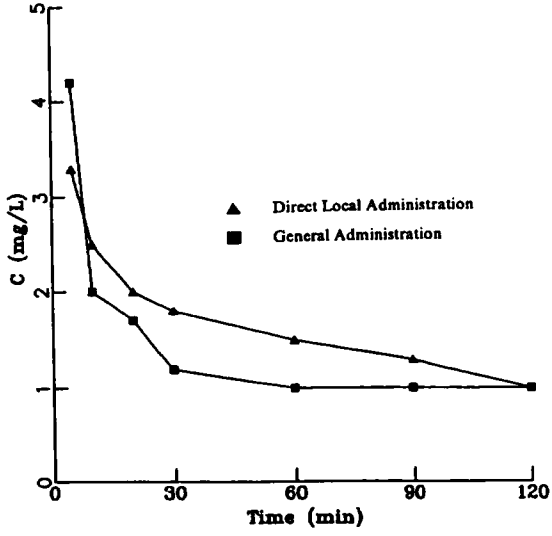


**Figure 5.** Eliminiation Curve of MMC in Dog's Plasma.

## CONCLUSION

We have developed a convenient direct injection HPLC method for the determination of MMC in biological fluids utilizing a column switching technique. Human and dog plasma samples were tested. The results show that this method is simple, rapid and accurate.

## ACKNOWLEDGMENTS

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## REFERENCES

1. E. J. Deluccia, M. Arunyanart, L. J. Cline Love, Anal. Chem., **57(13)**, 1564-1568 (1985).

2. M. Arunyanart, L. J. Cline Love, J. Chromatogr., **342**, 293-301 (1985).

3. J. Haginaka, J. Wakai, H. Yasuka, T.Nakagawa, Anal. Chem., **59(22)**, 2732-2734 (1987).

4. Y. Qu. P. Hu, P. L.Zhu, J. Lig. Chromatogr., **14**, 2755-2768(1991)

5. D. W. Armstrong, R. Q. Terrill, Anal. Chem., **51(13)**, 2160-2163 (1979).

6. J. V. Posluszny, R. Weinberger, Anal. Chem., **60(18)**, 1953-1958 (1988),

7. M. J. Koenigbauer, M. A. Curtis, J. Chromatogr., **427**, 277-285 (1988).

8. M. A. J. Van Opstal, F. A. L. Van der Horst, J. Chromatogr., **495**, 139-151 (1989).

9. Y. M.L I, S. X.J iang, L. R. Chen, Chromatographia, **34 (1-2)**, 63 - 66 (1992).

10. Y. M. LI, L. R. Chen, Y. Qu, J. Liq. Chromatogr., **16**, 2583 - 2594 (1993).

11. J. Der Hartich, W. J. Van Oort, M. C. Y. M. Bocken, H. M. Pinedo, Anal. Chim. Acta, **127**, 47-53 (1981).

12. U. R. Tjaden, J. P. Langenberg, K. Ensing, W. P. van Bennekom, E. A. de Bruijn, A. T. Van Oosterom, J. Chromatogr., **232**, 355-367 (1982).

13. M. J. Czejka, W. Jager, J. Chromatogr., **497**, 336-341 (1989).

14. T. Ohkubo, T. Nambara, J.Chromatogr., **527**, 41-446 (1990).

15. U. R. Tjaden, E. A. de Bruijn, R. A. M. Van der Hoeven, C. Jol, J. Van Der Greef, H. Lingeman, J. Chromatogr., **420**, 53-62 (1987).

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