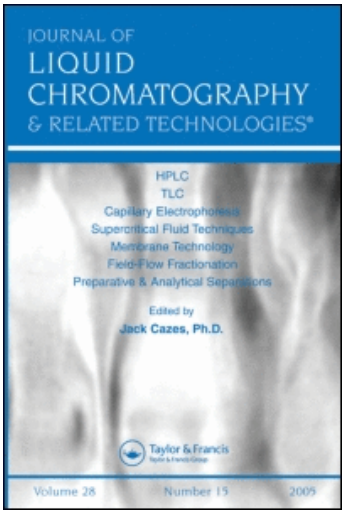

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Improved Liquid Chromatographic Method for Acyclovir Determination in Plasma

A. M. Molokhia[a]; E. M. Niazy[a]; S. A. El-hoofy[a]; M. E. El-dardari[a]
[a] College of Pharmacy King Saud University, Riyadh, Saudi Arabia

To cite this Article Molokhia, A. M. , Niazy, E. M. , El-hoofy, S. A. and El-dardari, M. E.(1990) 'Improved Liquid Chromatographic Method for Acyclovir Determination in Plasma', Journal of Liquid Chromatography & Related Technologies, 13: 5, 981 — 989
To link to this Article: DOI: 10.1080/01483919008049227
URL: http://dx.doi.org/10.1080/01483919008049227

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(5), 981-989 (1990)

IMPROVED LIQUID CHROMATOGRAPHIC METHOD FOR ACYCLOVIR DETERMINATION IN PLASMA

A. M. MOLOKHIA, E. M. NIAZY, S. A. EL-HOOFY, AND M. E. EL-DARDARI
College of Pharmacy
King Saud University
Riyadh, Saudi Arabia

ABSTRACT

A simple and reproducible method for determining acyclovir (ACV) in plasma is presented. The method involved the use of acetaminophen as internal standard. A single extraction step was performed using trichloroacetic acid for protein separation. After pH adjustment, samples from the supernatent layer were directly injected into a high pressure liquid chromatograph. Components separation was perfected through manipulation of solvent combinations and pH. The acyclovir and the internal standard retention times were 8.5 and 11 min. respectively. High correlation was obtained between AUC and the drug concentration ($r > 0.99$). Statistical analysis showed that the method is highly reproducible for ACV determination in aqueous solutions or in plasma. The mean drug recovery was better than 88%. The sensitivity obtained should enable the use of the method in future bioavailability and/or pharmacokinetic studies.

INTRODUCTION

Acyclovir, 9-(2-hydroxyethoxymethyl) guanine was the first non-toxic drug to be developed for systemic use against herpes group viral infections (1). Different preparations of the drug are available for parenteral, oral and topical administration. The oral route showed slow and poor absorption of the drug from the gastrointestinal tract (2). The percutaneous drug absorption appeared minimal when applied on intact skin. Acyclovir was not detected in blood or urine after seven days of four times daily topical application to intact human skin, of 25 mg drug in an ointment base (3).

Despite the apparent need for in-vivo research to improve drug bioavailability, the literature survey indicates limited efforts in this area. A simple and practical method for drug assay in biological fluids should encourage the widely needed research. The tritium labelled drug was used to follow its in-vitro absorption through human skin (4). The ^{14}C-labelled ACV was used to study the drug plasma protein binding (5). A radioimmunoassay method developed (6) and later validated (7) has been used to determine ACV in plasma and urine. Despite the high sensitivity of the previous methods, their wide use has been limited by impracticability and high expenses. HPLC has been used to study drug flux in-vitro (8,9). An HPLC method for ACV determination in serum (10) was found to be promising but not satisfactory for our needs of sensitivity and reproducibility.

In this communication a simple economical and reproducible method for determining ACV in plasma is described. Drug recovery was better than 88%.

EXPERIMENTAL

Instrumentation:

The following apparatus from Waters Associates (Milford, MA, USA) was used: a Model 6000 A Solvent delivery module; a Model 481 Variable

wave length detector; a WISP 710 B Autosampler injector and a Model 730 M Integrator. Chromatographic separations were performed using 10 µm µ-Bondapak-C_{18} column (30 cm X 3.9 mm i.d.).

Reagents:

Acyclovir powder (Wellcome Foundation Ltd., London, England) was tested for purity by TLC and was kept in a refrigerator. Acetaminophen, Phosphoric acid (BDH Chemicals Ltd., Poole, England), Trichoroacetic acid (E. Merck AG, Darmstadt, West Germany) and Sodium octane sulfonate (Waters BIC B-8 reagent, Waters Chromatography Division, Millipore Corporation, Milford, MA 01757 USA) were used as supplied. Methanol (E. Merck AG, Darmstadt, West Germany), was HPLC grade.

MObile Phase:

The mobile phase was prepared by dissolving 5 mM sodium octane sulfonate in 700 ml of double distilled water (DDW). The pH was then adjusted to 2.5 with phosphoric acid. Exactly, 70 ml of methanol were then added and the mixture was completed to 1 L with DDW. The mobile phase was daily prepared and degassed for 5 min. immediately before use.

Stock Solutions:

Stock solutions of acyclovir (100 ug/ml) and the internal standard (200 ug/ml) were prepared in DDW, protected from light and stored in a refrigerator for no more than one month. Solutions were equilibrated at room temperature directly before use.

Standard Curve:

Five sets of ACV solutions in the concentration range 0.5 - 7.5 µg/ml, containing the internal standard (5 µg/ml), were separately prepared. Exactly 10 µl of each solution were injected in duplicate onto the HPLC column. The ratios of the area under the curve were tabulated and plotted

versus ACV concentration. The results were statistically treated for evaluating the reproducibility of the method.

Determination of Acyclovir in Plasma:

Preliminary experiments with a number of candidate internal standards have shown that acetaminophen is well suited for this purpose. It was readily extracted from plasma alongwith ACV and when injected in the HPLC, it had a well distinct peak separate from that of ACV.

Human plasma was spiked with concentrated ACV and internal standard solutions to produce concentrations in the same range as those used in the standard curve. After mixing, 200 µl of trichloroacetic acid (98%) were added. The samples were then vigorously shaken on a vortex for one minute and centrifuged at 3000 rpm for ten minutes. About one ml of the supernatant was then taken and have its pH adjusted to 3 by 3 N sodium hydroxide solution (about 125 ul were used). Two samples each of 10 ul of the last solution were injected in the HPLC. The chromatographic conditions included: mobile phase flow rate of 1 ml/min.; prior equilibration of the column with the mobile phase was achieved by allowing the instrument to run for two hours before injecting the sample solutions; UV detector was set at 254 nm with optical density setting at 0.005 AUFS; and chart speed was maintained at 0.5 cm/min.

Acyclovir Recovery:

Five sets of plasma solutions spiked with ACV and the internal standard, in the same concentration range used in obtaining the standard curve, were prepared and extracted according to the method described above. The final extract with its pH adjusted to 3 was injected in duplicate for AUC determination. The results were compared with those obtained in absence of plasma and recovery was calculated.

RESULTS AND DISCUSSION

Fig. 1 shows typical chromatograms obtained when blank plasma, plasma containing internal standard and plasma containing ACV and internal standard were extracted and injected in the HPLC. As can be seen, good separation of the two compartments with no interference from plasma was observed. Retention times for ACV and the internal standard were 8.5 and 11 min respectively. After obtaining the ACV peak, the mobile phase was allowed to flow for 7 more minutes to ensure the complete washing of extracted residues. About 20 minutes were required for each run.

Table 1 and 2 present the results obtained in running the standard curve experiments.

Linear relationships were obtained when the ratio of the area under the curve of the drug to that of the internal standard was drawn against ACV concentration in each experiment ($r>0.99$). When the corresponding standard curves obtained after extracting the drug and the internal standard from plasma (Table 2) were compared to those obtained in the aqueous solutions (Table 1), identical slopes were observed.

The high reproducibility of the assay was established from statistically analysing the data in Tables 1 and 2. Analysis of variance, carried according to literature (11), showed that no significant difference existed among the runs in each Table ($F_1=0.065$, $F_2=0.039$).

Table 3 shows the results of ACV recovery as calculated from Tables 1 and 2 by dividing the ratio of AUC, obtained in plasma by the corresponding ratio obtained in aqueous solution.

The mean recovery values indicate that at least 88% of ACV were recovered from plasma. The percentage recovery increased as the drug concentration increased. Such high ACV recovery together with the high sensitivity (minimum detectable concentration in plasma = 0.1 μg/ml)

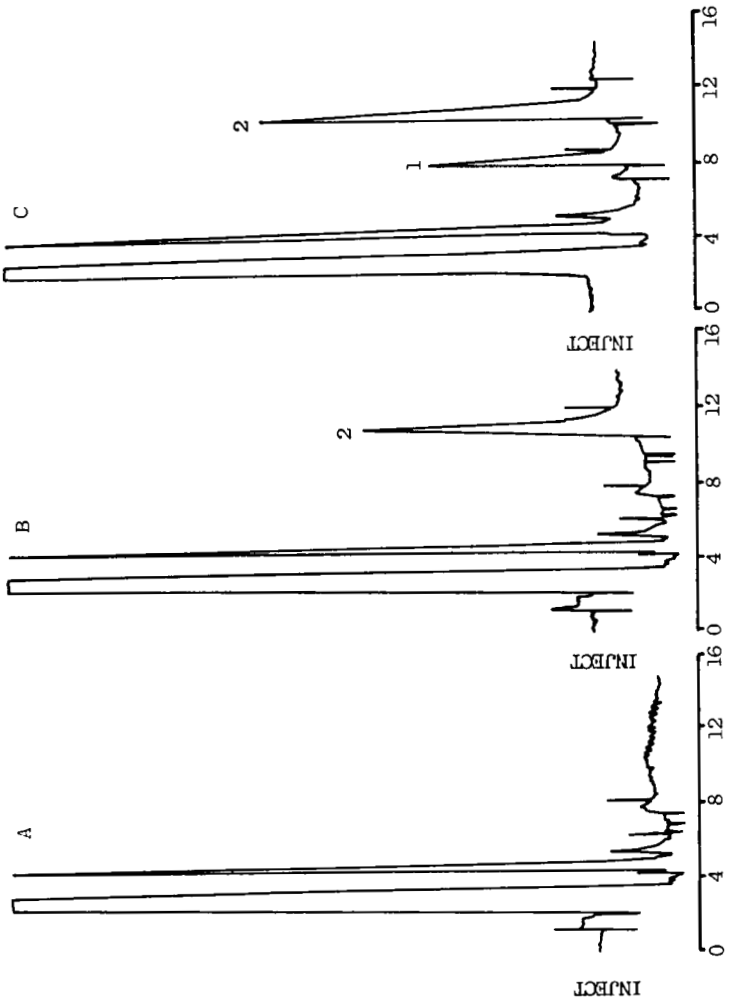

FIGURE 1: Typical chromatograms of blank plasma (A), Plasma containing internal standard (B), and plasma containing Acyclovir and the internal standard (C).

Key 1; Acyclovir, 2; Internal standard

TABLE 1

Ratio of AUC of ACV to that of Acetaminophen Obtained from Aqueous Drug Solutions

ACV Conc. (µg/ml)	AUC Ratio					Mean	± S.D.
	1	2	3	4	5		
0.5	0.134	0.122	0.100	0.180	0.145	0.136	0.026
1.25	0.355	0.310	0.322	0.308	0.330	0.325	0.017
2.5	0.683	0.541	0.605	0.550	0.655	0.606	0.056
3.75	1.010	0.940	0.928	0.920	0.957	0.951	0.032
5	1.413	1.234	1.266	1.255	1.240	1.281	0.066
7.5	2.133	1.543	1.96	1.969	1.855	1.892	0.195
Slope	0.142	0.103	0.13159	0.12956	0.12177	0.12574	
Y-intercept.	-0.0188	0.059	-0.0357	-0.0216	0.03151	0.00258	
r	0.99956	0.99371	0.99959	0.99677	0.99998	0.99980	

F value = 0.065

TABLE 2

Ratio of AUC of ACV to that of Acetaminophen Obtained from Spiked Plasma

ACV Conc. (µg/ml)	AUC Ratio					Mean	± S.D.
	1	2	3	4	5		
0.5	0.094	0.098	0.090	0.184	0.141	0.121	0.036
1.25	0.274	0.253	0.331	0.242	0.315	0.283	0.034
2.5	0.600	0.545	0.544	0.566	0.590	0.569	0.025
3.75	0.980	0.840	0.820	0.920	0.950	0.902	0.062
5	1.383	1.277	1.219	1.125	1.228	1.246	0.084
7.5	2.131	1.463	1.927	1.977	1.889	1.877	0.222
Slope	0.1470	0.10650	0.12890	0.12813	0.12509	0.12722	
Y-intercept.	-0.0941	0.03485	-0.05901	-0.0330	-0.0026	-0.03306	
r	0.99919	0.96544	0.99611	0.99232	0.9994	0.9904	

F value = 0.039

TABLE 3

ACV Recovery from Plasma

Conc. (μg/ml)	% Recovery					Mean	± S.D.
	1	2	3	4	5		
0.5	70	80	90	102	97	88	11.5
1.25	77	82	102	80	95	88	9.6
2.5	88	90	90	103	90	92.2	5.45
3.75	97	91	88	100	99	95	4.6
5	98	103	96	90	99	97	4.26
7.5	100	95	98	100	102	99	2.36

should make it possible to use this method in following the drug in human or animal plasma.

The method presented in this communication is simple. It is easily reproducible, once the pH of both the mobile phase and the injected solution is adjusted to the given values. A preliminary experiment carried on rabbit plasma after i.v. infusion with 20 mg/kg ACV showed that drug concentration was easily determined during the following six hours (about 2 half-lives). Previous studies on ACV pharmacokinetics in human (12,13) indicated that the therapeutic concentrations of the drug in plasma ranges from 0.1 - 6 μg/ml with most of the working concentrations above 0.5 μg/ml. This finding supports the possible use of the presented method of ACV analysis in human or animal studies.

ACKNOWLEDGEMENTS

The authors are grateful to the help provided by Dr. M.E. Abdulla in the statistical analysis and to Mr. K. Abbas for typing the manuscript. This research was supported by the Research Center, College of Pharmacy, King Saud University.

REFERENCES

1. Elion, G.B., Furman, P.A., Fyfe, J.A., Proc. Natl. Acad. Sci. USA. 74, 5716-5720 (1977).
2. Van Dyke, R.B., Connor, J.D., Wyborny, C., Am. J. Med., 73 (1A), 172 (1983).
3. Evoy Mc., Ed. C.K., Drug Information, American Society of Hospital Pharmacists, Bethesda, 1987.
4. Freeman, D.J., Sheth, N.V., and Spruance, S.L., Antimicrob. Agents and Chemother., 29 (5), 730 (1986).
5. De Miranda, P., Whitley, R.J., and Blum, M.R., Clin. Pharmacol. Ther., 6, 718 (1979).
6. Quinn, R.P., De Miranda, P., Gerald, L., and Good, S.S. Anal. Biochem., 98, 319 (1979).
7. Hintz, M., Quinn, R.P., Spector, S.A., Keeney, R.E., and Connor, J.D. Current Chemother, and Infectious Disease, 2, 1385 (1980).
8. Spruance, J.L., Mc Keough, M.B., and Cordinal, J.R., Antimicrob. Agents and Chemother., 25 (1), 10 (1984).
9. Cooper, E.R., Merritt, E.W., and Smith, R.L., J. of Pharm. Sci., 74 (6) 688 (1985).
10. Private Communication, Millipore Intertech. BP 307 78054 St. Quentinen Yvelines Cedes, France.
11. Abdulla, M., Sayed, A., Bener, A., and Al-Ohali, T., Int. J. Biomed. Comp., 22 (1), 65 (1988).
12. Petty, B.G., Whitley, R.J., Liao, S., Krasny, H.C., Rocco, L.E., Davis, L.G., and Lietman, P.S., Antimicrob. Agents and Chemother., 31 (9), 1317 (1987).
13. Sullender, W.M., Arvin, A.M., Diaz, P.S., Connor, J.D., Straube, R., Dankner, W., Levin, M.J., Weller, S., Blum, M.R., and Chapman, S., Antimicrob. Agents and Chemother., 31 (11), 1722 (1987).