Absorption and Disposition of Colloidal Drug Delivery Systems. I. High-Performance Liquid Chromatographic (HPLC) Analysis of a Cyclosporin Emulsion

Susan J. Corvari,[1] R. Gary Hollenbeck,[1] James Leslie,[1] Karen I. Plaisance,[1] and David Young[1,2]

Received November 21, 1989; accepted July 5, 1990

The amount of cyclosporin A in an oil-in-water emulsion drug delivery system was determined by HPLC. The direct extraction and analysis of an intact emulsion were compared to the analysis of a cracked emulsion and an olive oil solution of the drug. The intra- and interday variability for the intact emulsion was less than 10% from 35 to 150 μg/ml, with recovery of 94%. Comparison of the assay results obtained with the emulsion and the olive oil solution gave a highly correlated regression line with a small intercept and a slope close to unity. Thus, the direct extraction and HPLC analysis of drugs in emulsions may be a viable approach to evaluate drug content.

KEY WORDS: emulsion; high-performance liquid chromatography (HPLC); cyclosporin; drug delivery.

INTRODUCTION

During the last 10 years there has been interest in the use of emulsions for drug delivery (1–4). Drug absorption, distribution and clearance have been investigated after oral and parenteral administration of emulsion systems (5–8). The drug concentration within these emulsions, however, has been assumed to be the same as the concentration initially placed in the formulation.

Although the processing of the emulsion may result in a loss of drug by degradation or incomplete incorporation into the emulsion, few investigators have quantitated the actual amount of drug in emulsions. A stability indicating assay was developed for econazole nitrate cream by Christinat *et al.* (9) but the assay involved cracking the cream prior to the extraction procedure. Teagarden *et al.* (10) described an HPLC assay for a prostaglandin E_1 emulsion with direct injection of the emulsion onto the column without an extraction step. The surfactants in the emulsion, however, may shorten the life span of the column.

The purpose of this study was to determine if a reproducible HPLC method with liquid/liquid extraction could be developed for an oil-in-water (O/W) emulsion delivery system without cracking the emulsion. The HPLC procedure was used for both cracked and intact emulsions and the results were compared. Direct injection was not used in this study because of the effect of surfactants on the column.

MATERIALS AND METHODS

Materials

Cyclosporin A (lot 60845A) and cyclosporin D (lot 60845D) were gifts from Sandoz Pharmaceuticals, East Hanover, NJ. Olive oil was purchased from Sigma Chemical Company, St. Louis, MO. Sodium hydroxide, sodium phosphate monobasic, 1.0 *N* hydrochloric acid, 1.0 *N* sodium hydroxide, oleic acid, ether, hexane, acetonitrile, and methanol were purchased from J. T. Baker Chemical Company, Philipsburg, NJ. All chemicals were ACS analytical grade and all solvents were HPLC grade. Deionized water was prepared by the Milli-Q Water System Millipore, Milford, MA.

Sample Preparation

Olive Oil Standards. A solution of cyclosporin A, 0.1 mg/ml, was made by dissolving 10.0 mg of the drug in 100 ml of olive oil. From this solution standards were prepared ranging in concentration from 35 to 200 μg/ml.

Emulsion Standards. The oil phase of each emulsion consisted of cyclosporin A dissolved in olive oil and oleic acid, 26:1 (w/w). The aqueous phase was 0.07 *M* sodium hydroxide. The phase volume ratio of each emulsion was 0.5. The aqueous solution was added to the oil solution and stirred. When the two phases are mixed, the oleic acid and sodium hydroxide react to form sodium oleate, representing 2% (w/w) of the final emulsion. Emulsification was completed by passing the mixture through a Microfluidizer (Model 110, Microfluidics Corp.). The air pressure setting used for the Microfluidizer was 40 psi, and the time of emulsification 2 min. Five emulsions were prepared ranging in concentration from 3.5 to 20 mg/ml. One hundred microliters of each emulsion was diluted with 10 ml of deionized water to form emulsions ranging in concentration from 35 to 200 μg/ml. To confirm the colloidal nature of all emulsions, the mean particle size and particle size distribution were determined by photon correlation spectroscopy (Coulter Electronics, Hialeah, FL). The mean particle size and standard deviation of the particle size distribution ranged from 247 to 265 and from 79 to 81 nm, respectively.

Methanol Standards. The methanol stock solution was prepared by dissolving 10.0 mg of the drug in 100 ml of methanol. From this solution standards were prepared at 35 and 100 μg/ml.

Internal Standard. The stock solution of the internal standard was prepared by dissolving 10 mg of cyclosporin D in 10 ml of methanol. To prepare the working internal standard (WIS), 0.8 ml of the stock solution was diluted to 500 ml of water for a concentration of 1.6 μg/ml.

Extraction

Olive Oil Solution and Emulsion Samples. A volume of 50 μl from each of the standard cyclosporin A solutions and emulsions was placed in a disposable borosilicate test tube.

[1] Clinical Pharmacokinetics Laboratory, Department of Pharmaceutics, School of Pharmacy, University of Maryland, 20 North Pine Street, Baltimore, Maryland 21201.

[2] To whom correspondence should be addressed.

0724-8741/91/0100-0040$06.50/0

To one set of emulsion standards, 500 μl of 0.1 *N* HCl was added to crack the emulsion. The samples were mixed and centrifuged for 10 min. Five hundred microliters of 0.1 *N* sodium hydroxide was then added to each of these samples to neutralize the acid. To another set of emulsion standards the step to crack the emulsion was eliminated.

To all cracked emulsion, intact emulsion, and olive oil solution samples, 2 ml of WIS was added and then mixed. Cyclosporin A was extracted from all samples into 7 ml of ether by shaking on a horizontal shaker for 10 min and then centrifuging for 10 min. The ether layer was removed and evaporated to dryness at 40°C under a gentle stream of nitrogen. Samples were reconstituted with 2 ml of methanol, mixed, acidified with 1 ml of 0.025 *N* HCl, and mixed again. A volume of 7 ml of hexane was added to each sample which was then placed in a horizontal shaker for 10 min and centrifuged for 10 min. The hexane layer was aspirated. The hexane wash was repeated once to assure that all lipid components were removed. After neutralization with 0.1 *N* NaOH, samples were extracted back into ether, evaporated to dryness, reconstituted with mobile phase, and injected.

Methanol Samples. To 50-μl aliquots of each standard, 2 ml of WIS was added and then mixed. The standards were evaporated to dryness under a gentle stream of nitrogen at 40°C, reconstituted with mobile phase, and injected. The percentage recovery of the extraction procedure was determined for the olive oil solution by comparing the peak height ratios to the methanol solution peak height ratios at two concentrations, 35 and 100 μg/ml.

Chromatographic Conditions

The amount of cyclosporin in all samples was determined by HPLC. The method of Sawchuk and Carter (11) was used with a slight modification. The mobile phase consisted of 40% acetonitrile, 30% methanol, and 30% deionized water filtered through a 0.22-μm nylon filter (Rainin Instrument Company, Woburn, MA). The HPLC system consisted of a Model 6000A solvent delivery system (Waters Associates, Milford, MA), a Kratos Spectroflow 783 detector (Kratos Analytical, Ramsey, NJ) operated at 208-nm wavelength, and a Wisp 710B autosampler (Waters Associates). A 4.6 mm × 15-cm 5-μm Supelco LC1 column heated to 70°C was used. The mobile phase flow rate was 1.2 ml/min.

Precision and Accuracy

Cyclosporin standard curves for the intact emulsion, cracked emulsion and olive oil solution were generated (35 to 150 μg/ml) in triplicate on 3 different days. The intra- and interday assay precision for cracked and intact emulsion were determined by calculating the coefficient of variation (CV, %). The peak height ratio of cyclosporin A to cyclosporin D was plotted versus concentration to determine the linearity of each standard curve and the regression coefficient calculated.

To determine the relative accuracy of the HPLC procedure for intact emulsions, the concentrations from these emulsions were compared to those obtained for a cracked emulsion and an oil solution on 3 different days. Linear regression lines were calculated to determine if the intact emulsion was highly correlated to the cracked emulsion and the solution. The concentration range was increased to 200 μg/ml for this correlation study. In addition, intercept and slopes of the correlations were statistically compared to zero and unity by two-sided *t* tests (12).

RESULTS AND DISCUSSION

Typical chromatograms for intact and cracked emulsions are presented in Fig. 1. The retention times for cyclosporin A and cyclosporin D (approximately 8.55 and 10.57 min, respectively) were similar for all samples analyzed.

The standard curves for all samples were linear over the range of concentrations examined with no systematic error. A representative linear regression equation for the standard curves is

$$Y = 0.209 + 1.54*10^{-2}X$$

with a correlation coefficient of 0.998. The intra- and interday analysis of cyclosporin A emulsions in the presence and absence of 0.1 *N* HCl was shown to be reproducible, with coefficients of variation less than 10% at all concentrations studied (Table I).

The comparisons between the analysis of intact emulsion and olive oil solution or cracked emulsion for all 3 days were found to be highly correlated, with coefficients of determination of 0.985 and 0.989, respectively (Figs. 2 and 3). The regression equations defining these comparisons were

$$Y = 0.122 + 0.996\,X$$

for intact emulsion to olive oil solution and

$$Y = 0.450 + 0.933\,X$$

for intact emulsion to cracked emulsion. The intercepts close to zero and slopes close to unity indicate that the analysis of the intact emulsion is accurate and comparable to the analysis of olive oil solution or cracked emulsion. The intercept and slopes were not significantly different from zero and unity as determined by the *t* test at a 1% level.

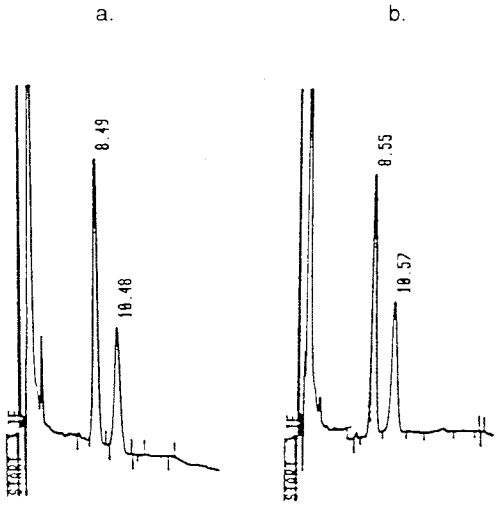

Fig. 1. Typical chromatograms for intact (a) and cracked (b) emulsions of cyclosporin A (approximate RT = 8.50 min), and cyclosporin D (approximate RT = 10.50 min). Injection volume = 50 μl and amount injected = 400 ng.

Table I. Intraday and Interday Assay Precision for Cyclosporin A in O/W Emulsions

	Concentration (μg/ml)	Amount (ng)[a]	Interday CV (%)[b]	Intraday CV (%)[b]
Cracked[c]	35	140	8.144	3.510
	50	200	3.612	1.666
	75	300	8.968	4.485
	100	400	9.165	3.690
	150	500	5.107	3.229
Intact[d]	35[e]	140	8.949	6.310
	50	200	4.686	5.203
	75[e]	300	2.304	0.802
	100	400	7.224	3.696
	150	600	1.565	7.019

[a] Amount injected onto the column.
[b] $n = 3$.
[c] 0.1 *N* HCl added.
[d] No HCl added.
[e] $n = 2$.

The relative recovery for 35 and 100 μg/ml of cyclosporin A in the olive oil solution was 99.42 ± 6.16 and 94.58 ± 4.57 (mean ± SD, $n = 3$), respectively. Since the recovery of cyclosporin A in olive oil was greater than 94%, the comparable analysis between intact emulsion and solution would also suggest that the recovery from the intact emulsion was greater than 94%.

Although this study does not represent an exhaustive investigation of the quantitative analysis of drugs in emulsions, this is one of the few reports on emulsified drug analysis. The ability to analyze an emulsion without cracking provides an easier approach to evaluating drug content, but the general utility of this approach needs to be documented.

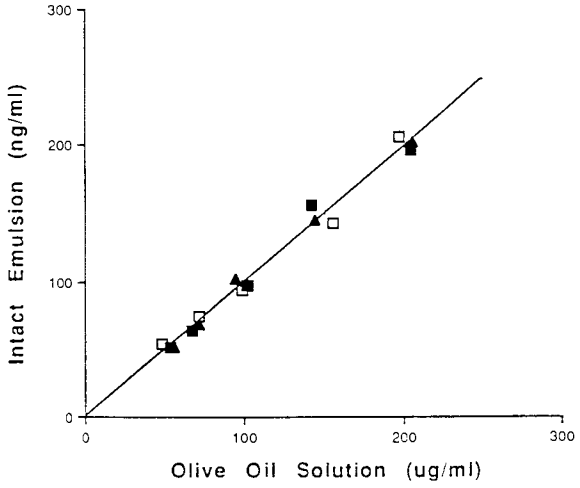

Fig. 2. A comparison of an intact emulsion to an olive oil solution of cyclosporin A on (▲) Day 1, (□) Day 2, and (■) Day 3.

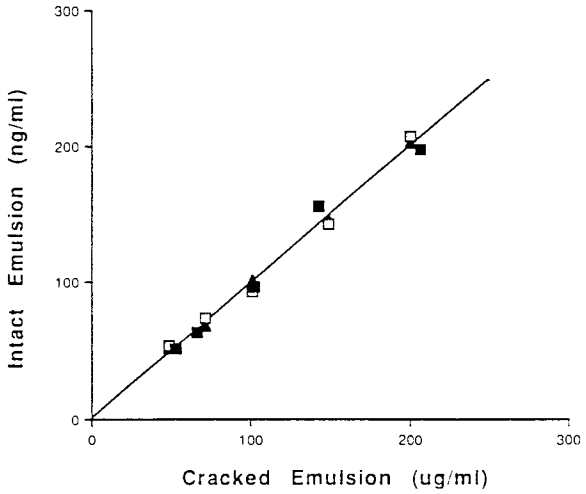

Fig. 3. A comparison of an intact emulsion to a cracked emulsion of cyclosporin A on (▲) Day 1, (□) Day 2, and (■) Day 3.

ACKNOWLEDGMENTS

This research was partially funded by Hoffman La-Roche, MediControl Corporation, the Designated Research Initiative Funds of the University of Maryland, and a NIH Basic Research Support Grant.

REFERENCES

1. M. Y. Levy and S. Benita. *Int. J. Pharm.* 54:103–112 (1989).
2. R. Kirsh, R. Goldstein, J. Tarloff, D. Parris, J. Hook, N. Hanna, P. Bugelski, and G. Poste. *J. Infect. Dis.* 158:1065–1070 (1988).
3. C. L. Fortner, W. R. Grove, D. Bowie, and M. D. Walker. *Am. J. Hosp. Pharm.* 32:582–584 (1975).
4. S. S. Davis. *Pharm. Tech.* 6:110–117 (1987).
5. B. D. Tarr and S. H. Yalkowsky. *Pharm. Res.* 6:40–43 (1989).
6. R. Jeppsson and S. Ljungberg. *Acta Pharmacol. Toxicol.* 36:312–320 (1975).
7. Y. Mizushima, T. Hamano, and K. Yokoyama. *J. Pharm. Pharmacol.* 34:49–50 (1982).
8. H. Sasaki, T. Kakutani, M. Hashida, and H. Sezaki. *J. Pharm. Pharmacol.* 37:461–465 (1985).
9. R. Christinat and H. W. Zulliger. *Drug Res.* 341:551–553 (1984).
10. D. L. Teagarden, B. D. Anderson, and W. J. Petre. *Pharm. Res.* 6:210–215 (1989).
11. R. J. Sawchuk and L. L. Carter. *Clin. Chem.* 27:1368–1371 (1981).
12. S. Bolton. *Pharmaceutical Statistics,* Marcel Dekker, New York, 1984, pp. 190–192.