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Short Communication

A comparison of disc and cartridge solid-phase extraction for the LC determination of rifampin and 25-desacetylrifampin in human serum

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1. Introduction

Rifampin, 3-[4-methyl-l-piperazinyl)imino] methyl]-refamycin SV, a semi-synthetic antibiotic drug, is widely used alone or in combination with other drugs such as isoniazid and pyrazinamide in the treatment of tuberculosis. Almost all of the LC methods published for rifampin (RIF) and its major metabolite 25-desacetylrifampin (DRI) (see Fig. 1) utilized classical liquid–liquid extraction of analytes from serum [1–4]. Other metabolites such as 3-formyl-25-desacetylrifamycin SV and 3-formylrifamycin SV are negligible in the plasma of healthy and ill patients [3]. Only one paper reported the use of an ethylsilane (C_2) solid-phase extraction (SPE) cartridge for sample preparation of RIF from serum [5]. There has been interest in this laboratory in the use of the recently introduced solid-phase extraction concentrator (SPEC) for sample preparation of the drug and metabolite in serum. The unique configuration of the SPEC is based on a silica-bonded phase which is uniformly distributed throughout a rigid glass microfiber disc [6]. The disc eliminates the channeling observed in conventional SPE cartridges and allows a significant reduction in bed mass, resulting in a highly efficient SPE method.

In this paper, a SPEC octylsilane (C8) disc is reported as the sample preparation step for the determination of RIF and DRI in human serum. Recoveries of both analytes were in the 98–99% range, and accuracy and precision data for the subsequent chromatographic run were within the 0.4–2.0% and 1.2–3.9% ranges, respectively. A comparison of the SPEC.C8 disc to a conventional C8 SPE cartridge showed increased recoveries of both analytes, and better accuracy and precision data, especially at the lower serum levels (0.5 μg ml $^{-1}$).

2. Experimental

2.1. Reagents and chemicals

RIF and DRI powders were obtained as gifts from Marion Merrell Dow (Kansas City, MO). Papaverine hydrochloride (internal standard) powder was purchased from Sigma (St. Louis, MO). Drug-free serum was purchased from Biological Specialty Corp. (Colmar, PA), and was used in the preparation of calibration curves and spiked serum samples. The SPEC.C8 discs (3 ml, 15 mg size) were obtained from ANSYS, Inc. (Irvine, CA), and the Bond-Elut brand of C8 SPE cartridges were purchased from Varian Sample Preparation Products (Harbor City, CA). All other chemicals and solvents were the highest grade of commercially available materials.

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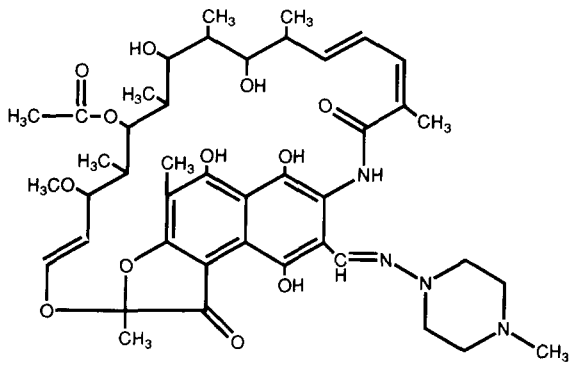
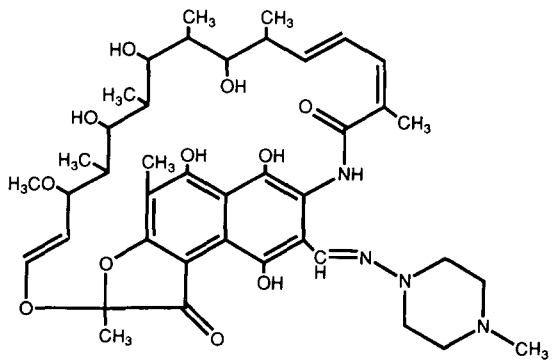
2.2. Preparation of stock and calibration solutions

A stock solution containing RIF (1 mg ml^{-1}) and DRI (1 mg ml^{-1}) was prepared by dissolving weighed amounts of each powder in dimethylformamide (DMF). In addition, a 50 μg ml^{-1} papaverine hydrochloride stock solution was prepared in water. Silylated glassware was not used in this study.

Aliquots of the stock solution were quantitatively transferred into volumetric flasks and diluted with acetonitrile–2 propanol (50:50, v/v) to volume, to obtain concentrations of 0.2, 2.0 and 20.0 μg ml^{-1} of each analyte. A 0.5 ml portion of each solution was then transferred into a disposable culture tube and evaporated to dryness at ambient temperature with the aid of a nitrogen stream. The residue was reconstituted with 200 μl of 5% ascorbic acid in 0.1 N hydrochloric acid solution, 100 μl of papaverine internal standard solution and 500 μl of blank human serum. After mixing well, the samples were applied to C8 disc and C8 cartridges for the sample preparation step.



RIFAMPIN



25-DESACETYLRIFAMPIN

Fig. 1. Chemical structures of rifampin and 25-desacetylrifampin.

2.3. Sample preparation step

The SPEC.C8 discs and the conventional C8 SPE cartridges were conditioned for serum samples with 2 × 1 ml acetonitrile followed by 2 × 1 ml 0.1 N hydrochloric acid. Serum samples (800 μl) were added to each disc and cartridge, and a vacuum was applied for 20 min. The analysis and internal standard were then eluted into 1 ml collection tubes with 5 × 100 μl portions of acetonitrile–methanol (60:40, v/v). After evaporation of the eluent to dryness at ambient temperature with the aid of a nitrogen stream, the residue was reconstituted in 300 μl of 0.1 N hydrochloric acid–water (67:33, v/v), and 50 μl was injected into the liquid chromatograph.

2.4. HPLC conditions

Chromatography was performed under isocratic conditions using system consisting of a Beckman Model 110B solvent delivery module (San Ramon, CA) and an ABI Model 759A UV/vis variable wavelength detector (Foster City, CA). The detector wavelength was set at 340 nm. Data acquisition was performed on a Hewlett-Packard Model 3392A Integrator (Avondale, PA).

The HPLC mobile phase and column used in this study was a modification of the procedure reported by Ishii and Ogata [3]. Separation was accomplished on a 5-μm Spherisorb ODS-1 column (250 × 4.6 mm i.d., Keystone Scientific, Inc. Bellefonte, PA) equipped with a direct-connect ODS guard column. The column was maintained at 40 ± 1 °C. The mobile phase consisted of acetonitrile–0.1 M monobasic potassium phosphate (pH 4.7; 0.1 M) (35:65, v/v) and was de-aerated by sonication before use. The flow-rate was set at 1.2 ml min^{-1}.

Quantitation was based on linear regression analysis of peak height ratios of each analyte to internal standard versus analyte concentrations.

3. Results and discussion

The mean recoveries of RIF and DRI from plasma using liquid–liquid extractions were reported to be 88 and 93%, respectively [3]. It

Table 1
Recoveries of RIF and DRI in Spiked Human Serum

Analyte	SPE device	% Recovery (mean ± SD) [a]	RSD (%)
RIF	Bond-Elut C2	97.8 ± 5.2	5.3
	Bond-Elut C8	87.4 ± 5.5	6.3
	Bond-Elut C18	74.2 ± 4.8	6.5
	SPEC.C8	99.0 ± 3.2	3.2
	SPEC.C18AR	79.2 ± 4.7	5.9
DRI	Bond-Elut C2	99.7 ± 11.2	11.2
	Bond-Elut C8	74.0 ± 6.9	9.3
	Bond-Elut C18	79.5 ± 3.5	4.4
	SPEC.C8	97.8 ± 5.7	5.8
	SPEC.C18AR	73.8 ± 4.5	6.1

[a] Based on triplicate recovery data at 0.5 and 20 μg ml^{-1} of each sample.

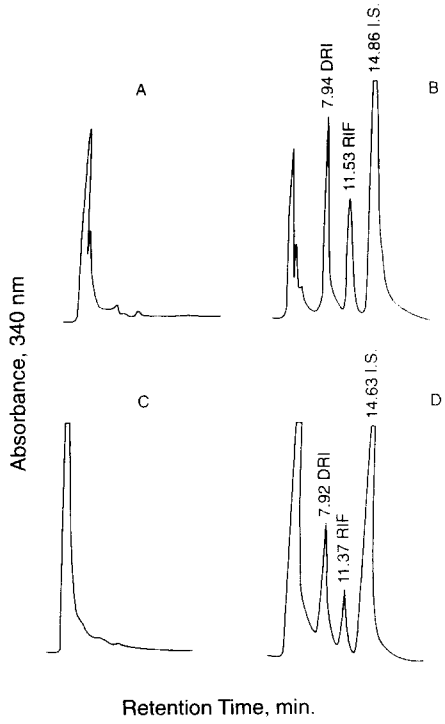


Fig. 2. Typical HPLC chromatograms of RIF–DRI assay showing: (A) blank serum and (B) spiked serum using SPEC.C8 disc; (C) blank serum and (D) spiked serum using conventional C8 SPE cartridge. The internal standard (IS) used was papaverine hydrochloride.

was of interest in this laboratory to study the recoveries of RIF and DRI from serum using conventional SPE and to compare the recoveries to those obtained with the recently introduced disc which may demonstrate advantages. Based on a typical oral dose of 450 mg of RIF to humans, plasma levels of RIF and DRI are typically in the range 0.1–20 and 0.1–3 μg ml^{-1}, respectively [3]. The recoveries of RIF and DRI from human serum were assessed for the SPEC.C8 disc and conventional C8 SPE cartridge using spiked samples at 0.5 and 20 μg ml^{-1} levels. The results shown in Table 1 indicate that higher recoveries for both analytes were obtained on the C8 disc compared to the C8 cartridge. It was of interest to note that comparable recoveries to the C8 disc were obtained using a conventional C2 cartridge. The recovery of papaverine (internal standard) on the SPEC.C8 disc was approximately 88%. Other SPEC discs such as SPEC.C18 and SPEC.MP1 (mixed phase) and a conventional C18 cartridge were also assessed for recoveries of RIF and DRI. There was no recovery observed for either analyte on the mixed-phase disc. None of the other commer-

Table 2
Accuracy and precision of RIF and DRI in spiked serum samples using C8 disc and conventional C8 cartridge

Analyte	SPE	Concentration added, (μg ml^{-1})	Concentration found, (μg ml^{-1}) [a]	Percentage error (accuracy)	RSD (%)
RIF	Bond-Elut C8	0.5	0.53 ± 0.11	6.0	21
		6.0	5.74 ± 0.18	4.3	3.1
		10.0	10.36 ± 0.51	3.6	4.9
	SPEC.C8	0.5	0.51 ± 0.02	2.0	3.9
		6.0	6.09 ± 0.19	1.5	3.1
		10.0	10.04 ± 0.12	0.4	1.2
DRI	Bond-Elut C8	0.5	0.57 ± 0.08	14	14
		6.0	6.35 ± 0.12	5.8	1.9
		10.0	10.29 ± 0.82	2.9	8.0
	SPEC.C8	0.5	0.51 ± 0.01	2.0	2.0
		6.0	5.95 ± 0.16	0.8	2.7
		10.0	9.88 ± 0.15	1.2	1.5

[a] Based on $n = 3$.

cially available stationary phases of the disc or cartridge were evaluated for use in the assay.

Table 2 shows accuracy and precision data for spiked concentrations of RIF and DRI in serum using both the C8 disc and C8 cartridge as the sample preparation step. The data is generally comparable, but the C8 disc is better at lower concentrations of RIF and DRI (0.5 $\mu g\ ml^{-1}$).

Fig. 2 shows representative HPLC chormatograms of the RIF-DRI assay using the C8 disc and C8 cartridge. The chromatogram obtained from the C8 disc is slightly cleaner with respect to a blank serum compared to that obtained with the C8 cartridge.

4. Conclusion

The value of the SPEC.C8 disc as an alternative to the conventional SPE cartridge in the sample preparation step of the HPLC assay of RIF and DRI was clearly demonstrated with accuracy in the 0.4–2.0% range and precision in the 1.2–3.9% range.

Acknowledgment

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