

EXTRACTION AND PURIFICATION OF CYCLOSPORINE FROM CAPSULE DOSAGE FORMFarah Aziz Khan^{*1}, Mohd. Fareed Khan² and A. S Thakur¹¹Department of Biochemistry, Govt. Medical College, Jagdalpur, Chattisgarh, India.²Department of Microbiology, Govt. Medical College, Jagdalpur, Chattisgarh, India.

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ABSTRACT

In the current research cyclosporine is extracted and purified for being used as a working standard in case of unavailability of reference standard. 5 gelatinous capsules of Panimun Bioral were cut and dissolved in ACN and 0.1% TFA. Solution was put in Preparative HPLC and RP-HPLC systems for qualitative and quantitative estimation of the drug in a gradient run of 25 min. Grace Vydac column was taken as stationary phase, and mobile phase was the mixture of ACN and TFA. Ultraviolet absorbance at 215 nm was set to detect cyclosporine. Chromatograms show the good intensity peak at the retention time 14 min. For confirmatory identification of molecular weight of the compound, Liquid Chromatographic Mass Spectroscopy was done, which results a peak of molecular weight 1202, representing cyclosporine. Purity of extracted cyclosporine was checked again in Reverse phase HPLC. Grace Vydac column was taken as stationary phase with ACN and TFA as mobile phase. Chromatogram shows the peak at correct retention time with high intensity and maximum peak area of 7508049 AU. Thus, by going through various chromatographic techniques, the purity of extracted compound was found to be 95.42% and was considered as working standard.

Keywords: Cyclosporine, RP-HPLC, chromatography, retention time, working standard.**INTRODUCTION**

Cyclosporine is a cyclic undecapeptide with a relative molecular mass of 1202.6.¹ Cyclosporine is a drug with immunosuppressive activity.² It is very useful in combating graft rejection while organ transplantation. It is thought to bind to the cytosolic protein, cyclophilin.³⁻⁶ Cyclosporine is distributed largely outside the blood volume and in blood the distribution is concentration dependent. The aqueous solubility of cyclosporine is 27.67 µg/ml at 25°C.^{7,8} It is extensively metabolized by CYP3A4, a subfamily of cytochrome p450.^{9,10} Several liquid chromatographic procedures have been reported for the analysis of cyclosporine. Some procedures require labor-intensive multistep liquid-liquid extractions to isolate cyclosporine from physiological fluids¹¹⁻¹³ and in some solid-phase extraction procedures, analytical recovery is poor and are prone to interferences from late-eluting peaks.¹⁴⁻¹⁶ The manuscript comprises of both qualitative and quantitative chromatographic techniques applied for the extraction and purification of cyclosporine from capsule dosage form.

MATERIALS AND METHODS

Soft gelatinous capsules of cyclosporine (Panimun Bioral by Panacea Biotech Ltd., India), 100 mg each, as labeled, were procured from local market. Its major components are hydrophilic solvent, a surfactant with high HLB values, a lipid and a hydrophobic drug. Acetonitrile (ACN) HPLC grade was purchased from Merck and Tri-fluoro acetic acid

(TFA) from Qualligens. HPLC grade water was obtained by double distillation and purification through milliQ water purification system.

To the 20 ml of ACN, 5 capsules of cyclosporine were cut and dissolved. To extract the desired compound, the solution was subject to preparative HPLC of system Waters. Grace Vydac (100 * 19mm) column was taken with mobile phase of ACN and 0.1% TFA in a gradient runs of 25 min. Flow rate was kept at 1 ml/min. Sample was injected at fixed loop of 20 µl and monitored at 210 nm. Six fractions were collected.

Reverse phase HPLC (RP-HPLC) of each fraction was done to ensure the absorbance of collected fractions. Column Grace Vydac (100*19mm) was used with mobile phase ACN and phosphate buffer of pH 7 in a gradient run of 35 min, with flow rate of 1 ml/min. Fractions whose peak appeared at retention time (RT) 14 min with absorbance of 0.80-1.20 were mixed altogether. Quantification of cyclosporine was based on RT and peak height.

Finally, to confirm the mass of eluted compound, liquid chromatographic-mass spectroscopy (LC-MS) was done. 0.1% TFA and ACN were taken as mobile phase in an isocratic run (20:80) at 1 ml/min. Instrument parameters were set as cone voltage 25V, capillary voltage 3.5V, ion energy 10.5 eV, ionization mode ES+, wavelength 200-400 nm and mass range 500-1500. Mass spectra revealed the highest peak of molecular weight 1202, i.e. of cyclosporine. The study was proceeded to check the purity of the extracted compound by RP-HPLC. 10 mg extracted cyclosporine was diluted in 10 ml solution of TFA and CAN in 1:1 ratio, and subject to a gradient run of 25 min. ACN and TFA buffer limit was set at 210 nm.

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RESULTS AND DISCUSSION

Through the preparative technique, six fractions were obtained from 20 ml solution. The fractions were run in RP-HPLC and those with good absorbance at RT 14 min were mixed (Figure 1). LC-MS spectra revealed the peak of molecular weight 1202, showing the extracted compound was cyclosporine (Figure 2). Purity of the compound was further checked by RP-HPLC; chromatogram revealed the peak of maximum area, i.e. 7508049 AU, at correct RT of 14.10 min, thereby concluding the purity of compound to be 95.42% (Figure 3, Table 1).

Table 1. Showing the purity of Cyclosporine

Peak No.	Retention Time (min)	Area (AU)	% Area
1	9.527	71733	0.91
2	11.752	70026	0.89
3	13.616	21864	2.78
4	14.108	7508049	95.42

Figure 1. RPHPLC chromatograms of max absorbance showing fractions

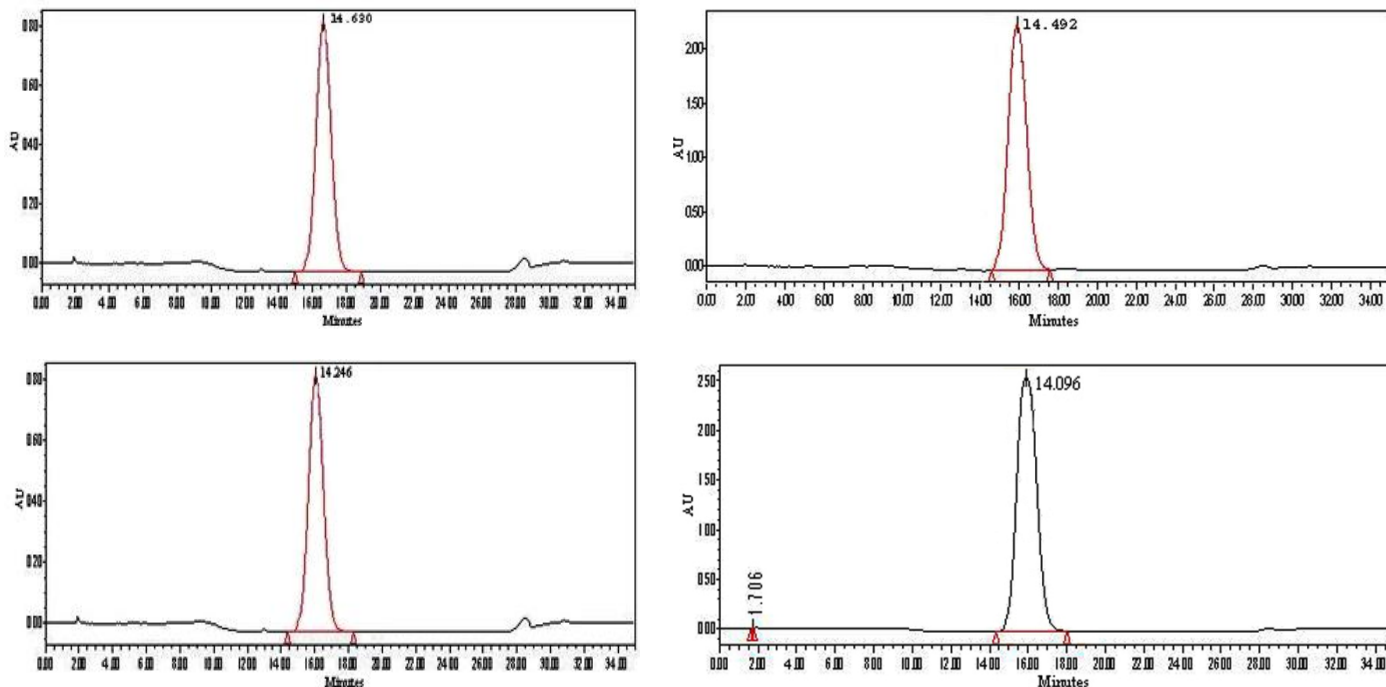


Figure 2. Mass spectra of mixed fractions, showing Cyclosporine

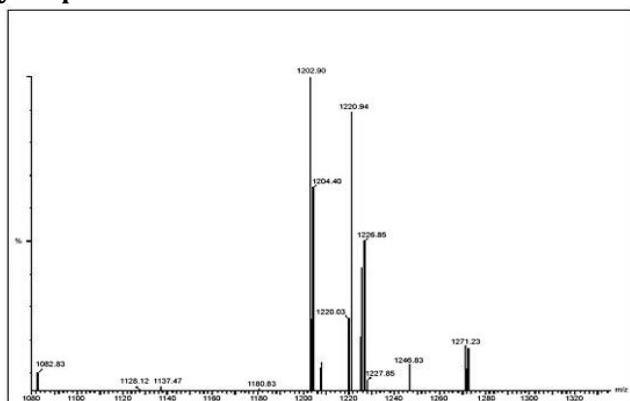
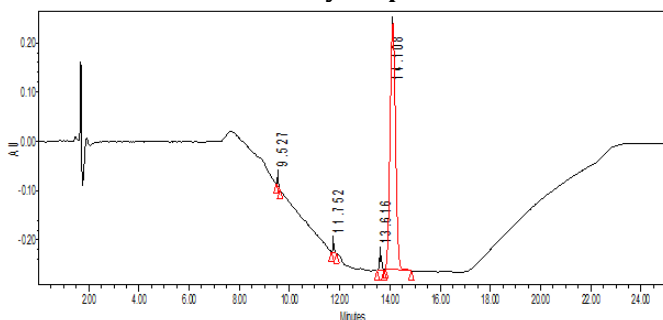


Figure 3. RP-HPLC Chromatogram shows the peak of max area at correct RT of cyclosporine



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The optimum conditions kept for the extraction of cyclosporine are described above. TFA used in this study results as an ion pairing agent and has low absorption within detection wavelength¹⁷ also played an important role in eluting the peak at correct time and unknown endogenous peaks did not interfere with cyclosporine. Ultra Violet (UV) spectrum of cyclosporine was recorded by scanning in the range of 200-400nm. At the wavelength of 210 nm, cyclosporine show good maxima. Similarly, ACN is also a suitable organic solvent for cyclosporine to get dissolved, and Grace Vydac column was found well satisfactory in separating cyclosporine from excipients peak.

CONCLUSION

The main purpose of this study was to consider the extracted and purified cyclosporine as a working standard in case when reference standard is not available. Chromatograms of preparative HPLC, RP-HPLC and LC-MS showed the cyclosporine peak of good intensity at correct retention time with accurate molecular weight. Thus, with high percent of purity, extracted cyclosporine can be used as working standard.

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