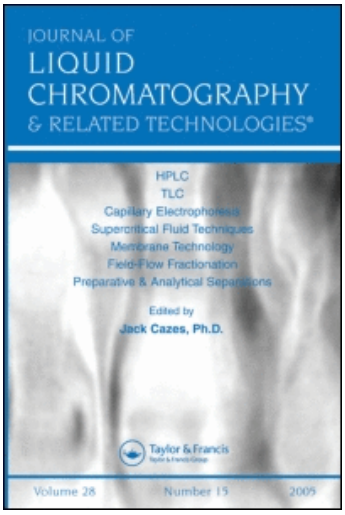

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Quantification of Acarbose in Human Plasma by Liquid Chromatography—Electrospray Tandem Mass Spectrometry

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ABSTRACT

The method for the determination of acarbose in human plasma is described, using high-performance liquid chromatographic separation with tandem mass spectrometric detection. Samples were prepared using solid phase extraction and separated on a Zorbax SB C18 column with a mobile phase consisting of water, acetonitrile, and triflouroacetic acid. Detection was performed by a TSQ quantum mass spectrometer in the selected reaction monitoring (SRM) mode using electrospray ionization (ESI). The method has a chromatographic elution time of 3 min and was linear within the range of 100–1000 ng/mL. The intra- and

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inter-run accuracy and precision, calculated from quality control (QC) samples, was less than 11%.

Key Words: Acarbose; LC/MS/MS; Plasma; Fexofenadine.

INTRODUCTION

Acarbose[1] is an oral alpha-glucosidase inhibitor for use in the management of type 2 diabetes mellitus. Acarbose is an oligosaccharide, which is obtained from the fermentation process of a microorganism, *Actinoplanes utahensis*. It is chemically known as *O*-4,6-dideoxy-4-[[(1*S*, 4*R*, 5*S*, 6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclo-hexane-1-yl]amino]-K-D-glucopyranosyl-(1→4)-*O*-K-D-glucopyranosyl-(1→4)-D-glucose (Fig. 1).

Only one method is reported for evaluating the effect of acarbose on the digestion of starch in rats by determining the ^{13}C-glucose.[2] No method for the direct determination of acarbose from human plasma is reported. This paper describes the development of a liquid chromatographic method, coupled with tandem mass spectrometry (LC/MS/MS) with electrospray ionization (ESI), for the quantitative determination of acarbose from human plasma using fexofenadine (Fig. 2) as an internal standard (IS).

EXPERIMENTAL

Materials

Acarbose was purchased from Sigma Pharma. Fexofenadine, human control plasma was received from Wockhardt Research Centre (Aurangabad,

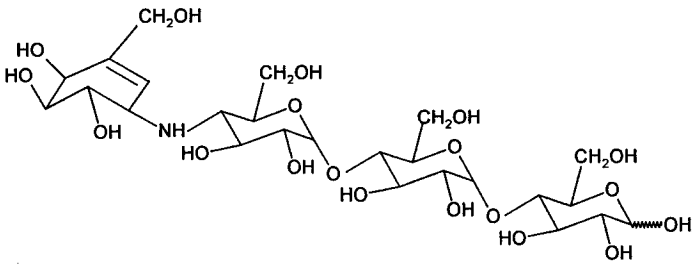

Figure 1. Chemical structure of acarbose.

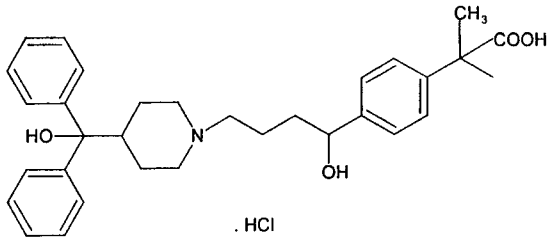

Figure 2. Chemical structure of fexofenadine.

India). Other reagents and solvents were of HPLC grade purchased from Merck Limited (Mumbai, India).

Preparation of Standard Solutions

Stock solutions of acarbose and fexofenadine were prepared in diluent (water : acetonitrile 50 : 50 v/v) at a free base concentration of 10,000 ng/mL. These stock solutions were appropriately diluted with diluent to prepare working standard solutions to obtain a calibration curve.

Preparation of Calibration Standard

Plasma samples for the calibration curve were prepared by adding 50 μL of the appropriately diluted standard solution of acarbose and 50 μL of internal standard (IS) (20 ng/mL) to 1 mL plasma. These samples were vortexed for 30 sec and processed by using an extraction manifold (Waters, USA). An Oasis HLB extraction cartridge (1 cc, 30 mg) was used for solid phase extraction. The extraction procedure was as shown in Table 1.

LC/MS/MS Analysis

A surveyor chromatographic pump and autosampler (Thermo Finnigan, UK) were used for all HPLC analyses. Separation was carried out on a Zorbax SB C18 analytical column (150 × 4.6 mm^2 i.d., 5 μm). The mobile phase was water, acetonitrile, and trifluoroacetic acid (25 : 75 : 0.005 v/v) at the flow rate of 0.5 mL/min.

The mass spectrometry was performed using a TSQ Quantum triple-stage quadrupole mass spectrometer with an ESI ion source (Thermo Finnigan, San

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Table 1. Solid phase extraction procedure for plasma samples.

Condition	Add to and draw through each cartridge 1 mL of methanol.
Equilibrate	Add to and draw through each cartridge 1 mL of water.
Dry	Dry each cartridge for 1 min.
Load	Add to and draw through each cartridge 1 mL of plasma sample.
Dry	Dry each cartridge for 1 min.
Wash	Add to and draw through each cartridge 1 mL of hexane.
Dry	Dry each cartridge for 1 min.
Release vacuum, remove manifold cover, and discard waste fluids. Insert rack containing collecting vessels, replace cover, and turn on vacuum.	
Elute	Add to and draw through each cartridge 1 mL of mobile phase, collecting elutes in suitable vessels and inject.

Jose, CA) in positive-ion selected reaction monitoring (SRM) mode. The sheath gas pressure was 23 psi and auxiliary gas pressure was 20 psi.

The mass spectrometer was programmed to transmit the precursor ions through the first quadrupole (Q1) to second quadrupole (Q2), where the ions underwent collision-induced fragmentation, to Q3, where the product ions were monitored. A collision gas pressure of 1.5 m Torr was employed and the collision offset was −15 V for this analysis. Two different scan events were used for acarbose and IS. The SRM parameter for these scan events was as shown in Table 2. Results were processed by using Xcaliber software (3.1).

Validation

The method was validated for accuracy and precision by assaying the acarbose quality control (QC) samples of 200, 500, and 900 ng/mL. The QC values were calculated from a standard regression curve, composed of nine different concentrations spanning the concentration range 100–1000 ng/mL for

Table 2. The selected reaction monitor parameters for acarbose and internal standard.

Parent	Product	Width	Time	CE	Q1PW	Q2PW	Tube lens
Scan event 1							
646.3	304.1	1.0	0.5	28	0.7	0.7	125
Scan event 2							
502.4	466.3	1.0	0.5	32	0.7	0.7	110

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acarbose. Data calculation was accomplished with an online computer system. Calculation of drug concentrations in unknown samples was based on a weighted $(1/x^2)$ least-squares regression of plasma calibrator concentrations against the peak area ratios. The peak area ratios were obtained by dividing the peak area of acarbose by the peak area of the IS. Each calibration curve must have a coefficient of determination of at least 0.99. Both the relative error and the relative standard deviation of each level of the linearity curves and QC samples must be $<15\%$ of the theoretical value, except for those at the limit of quantification, which must be $<20\%$.

RESULTS AND DISCUSSION

Development

Acarbose is an oligosaccharide, it has no response to a UV detector. Initially estimation of acarbose was tried by using HPLC with an refractive index (RI) detector, but there was too much interference from plasma peaks. Due to this, it becomes very difficult to quantitate the acarbose in nanogram levels. So, further attempts for the estimation of acarbose in plasma were made by using LC–MS/MS. Acarbose is highly water soluble and insoluble in most of the organic solvents, therefore the liquid–liquid extraction method did not work. A plasma deproteination method was tried, but it increased the background noise and, also, the backpressure of analytical column. Solid phase extraction gave clean samples and the time required for extraction is much less. Due to the high specificity and selectivity of MS/MS detection, no interfering or late eluting peaks were found when assaying blank plasma extracts from six different sources.

Full-scan positive-ion mass spectra of acarbose and IS predominantly showed the protonated molecular ion $(M + H)^+$ at 646.1 and 502.2, respectively. The product ion spectra of the protonated molecular ion of acarbose and IS are shown in Figs. 3 and 4, respectively. The ions at m/z 303.9 and 145.9 are derived from acarbose and those at 484.2, 466.2, and 170.98 from the IS. From this result, the mass spectrometer was set as follows: m/z 646.1 for acarbose and m/z 502.2 for the IS as the precursor ions, and m/z 303.9 for acarbose and m/z 466.2 for IS as the product ions in the SRM mode. The chromatographic elution time was 3 min. (Fig. 5).

Standard Curve Characteristics, Precision, and Accuracy

The calibration curves were linear in the studied range. The slope, intercept, and correlation coefficient (mean $\pm$ standard deviation) of standard

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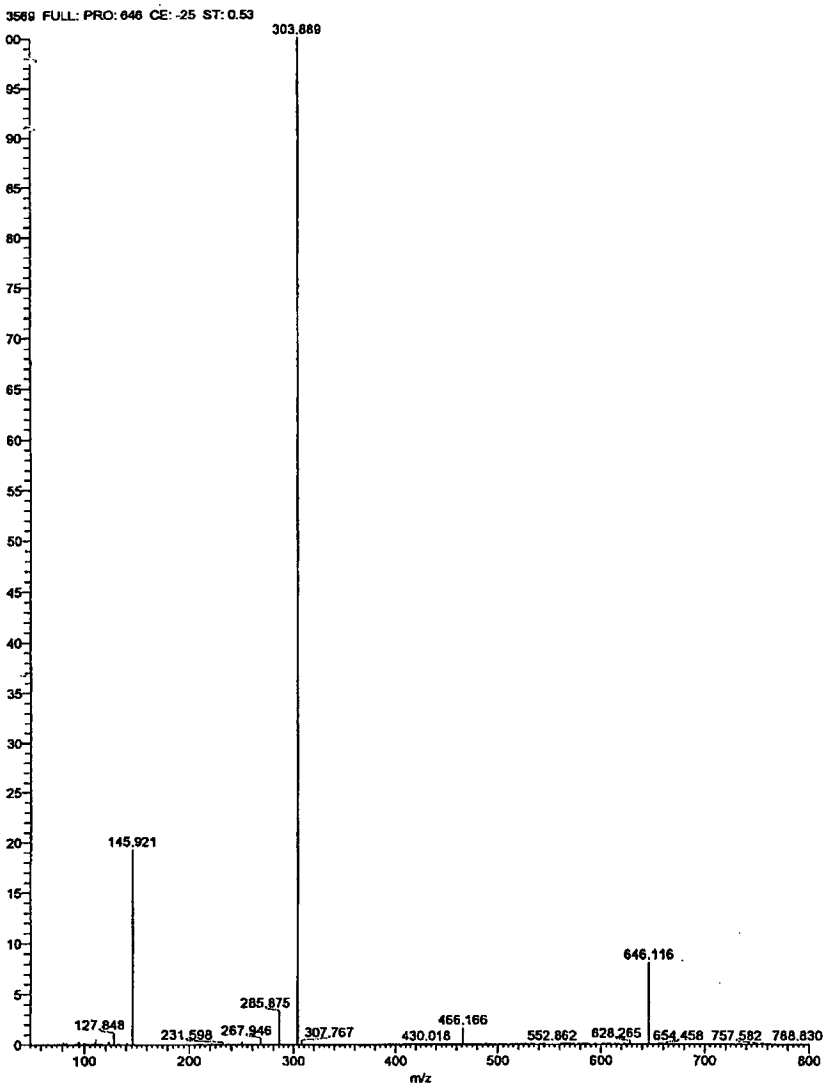

Figure 3. Product ion mass spectra in the positive-ion mode of the protonated molecular ion of acarbose.

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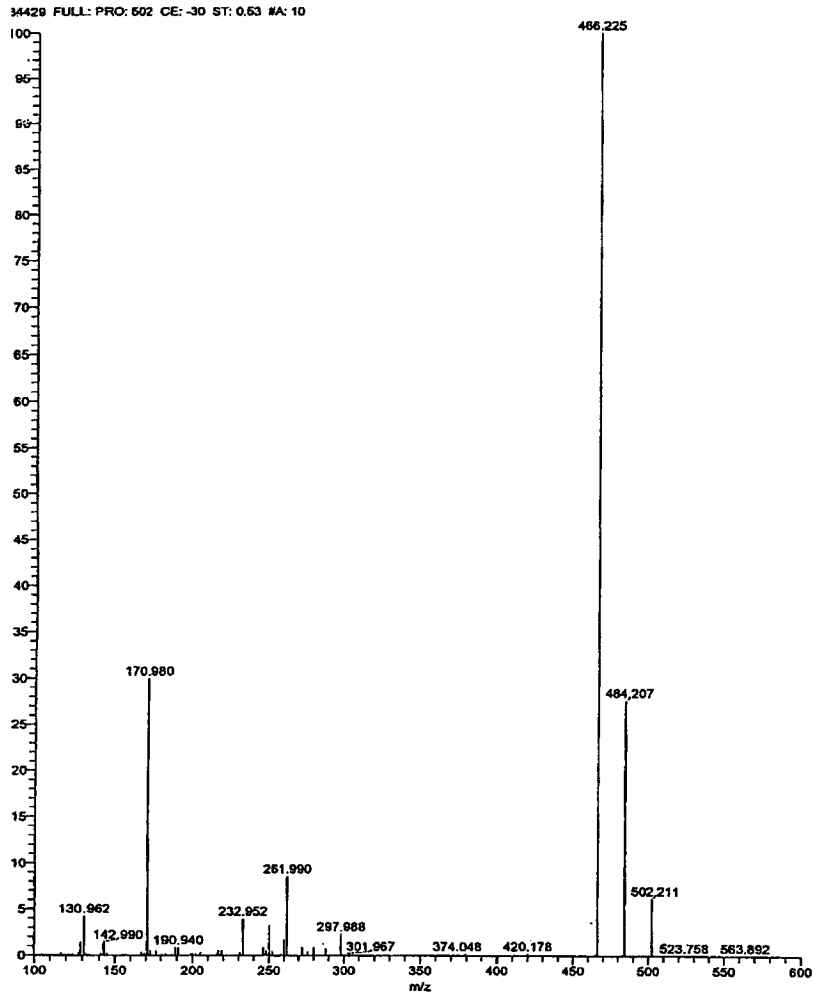

Figure 4. Product ion mass spectra in the positive-ion mode of the protonated molecular ion of fexofenadine.

curves ($n = 3$) were 0.0012982 ± 0.000015, -0.0364635 ± 0.01613, and 0.9930 ± 0.00378, respectively.

The back calculated concentration values for each level of acarbose were obtained from each calibration curve used during method validation. The obtained mean ($n = 5$) back calculated values, as well as the precision and accuracy estimations for each concentration level, are summarized in

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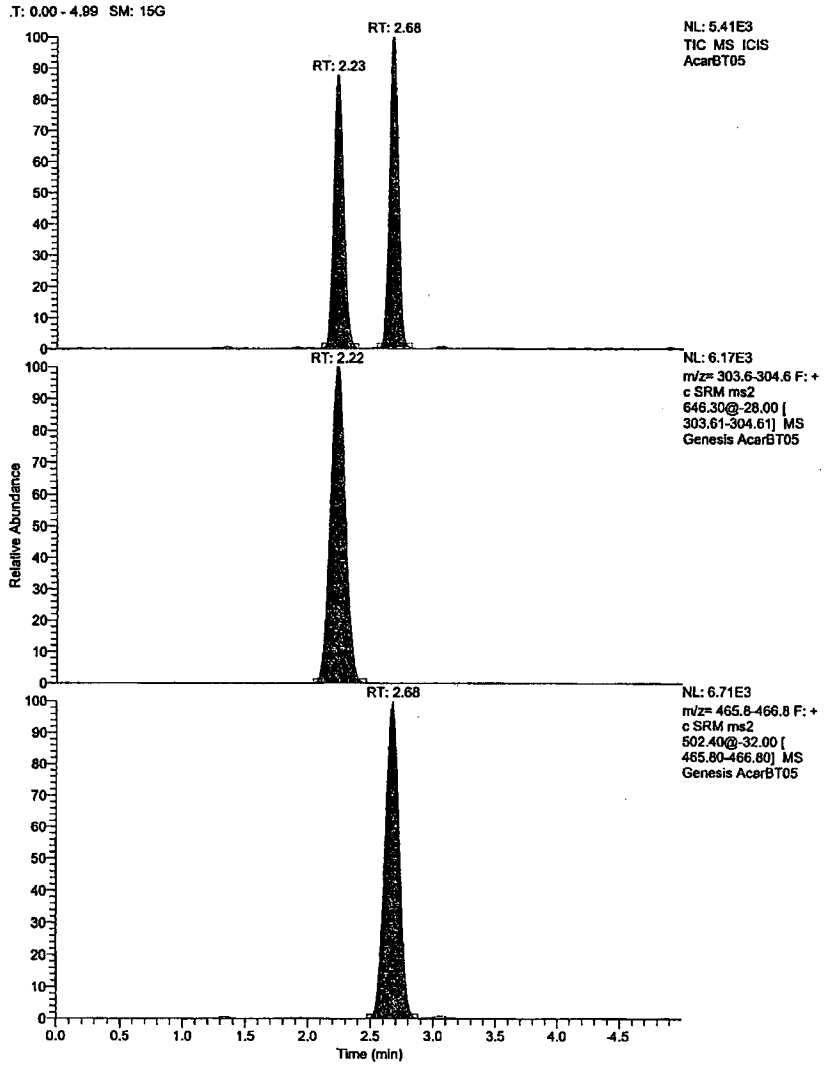

Figure 5. Chromatogram showing the peaks of acarbose and fexofenadine.

Table 3. The RSD and RE were 6.2% and 2.08%, respectively. The precision and accuracy for determination of acarbose was evaluated by replicate analysis of the QC samples at three different concentrations within the standard curve range (Table 4). Intra-day precision and accuracy was found to be less than 10.5%, and inter-day precision and accuracy was less than 10.9%.

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Table 3. Standard curve characteristics for acarbose.

Target value (ng/mL)	Mean ($n = 3$, ng/mL)	SD (%)	RE (%)	RSD (%)
100	96.65	1.21	−3.35	1.25
200	208.88	5.97	−4.44	2.86
300	323.86	19.51	7.95	6.02
500	479.81	24.10	−4.04	5.02
600	583.49	43.29	−2.75	7.42
700	700.88	89.53	0.13	12.77
800	791.83	83.15	−1.02	10.50
900	889.88	58.51	−1.12	6.58
1,000	997.63	37.23	−0.24	3.73

Note: SD, standard deviation; RE, relative error; RSD, relative standard deviation.

Table 4. Accuracy and precision for acarbose.

		Target concentration (ng/mL)		
		200	500	900
Day 1[a]	Mean	207.87	509.71	975.37
	SD	21.84	29.68	37.62
	%RE	3.94	1.94	8.37
	%RSD	10.51	5.82	3.86
Day 2[a]	Mean	198.82	478.04	912.04
	SD	15.82	48.13	68.74
	%RE	−0.59	−4.39	1.34
	%RSD	7.96	10.07	7.54
Day 3[a]	Mean	198.97	481.92	898.80
	SD	8.32	45.95	72.19
	%RE	−0.52	−3.62	−0.13
	%RSD	4.18	9.53	8.03
Overall[b]	Mean	201.89	489.89	928.74
	SD	15.71	41.59	66.63
	%RE	0.94	−2.02	3.19
	%RSD	7.78	8.49	7.17

[a]$n = 5$ determinations.
[b]$n = 15$ determinations.

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Stability Study

Freeze–thaw stability was carried out at three different concentrations, 200, 500, and 900 ng/mL. The plasma samples were stored at −40°C and subjected to three freeze–thaw cycles. The percentage deviation from the initial concentration was less than 9%, indicating no significant substance loss during repeated thawing and freezing.

Processed sample stability was carried out at three different concentrations, 200, 500, and 900 ng/mL. The samples were kept in an autosampler at ambient temperature and analyzed with regular intervals up to 24 hr. The percentage deviation from the initial results was less than 11.6%. The processed samples were found to be stable at ambient temperature for 72 hr.

CONCLUSION

The LC/MS/MS method for the determination of acarbose in human plasma has been developed and validated. It has been shown to be rapid, accurate, and precise. The statistical analysis of the precision and accuracy data demonstrates that the method is suitable for quantifying the analyte during clinical trials and/or pharmacokinetic studies.

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REFERENCES

1. Kelly, G.L., Murray, L., Sifton, D.W., Eds. *Physicians Desk Reference*, 57th Ed.; Medical Economics Company, Inc.: Montvale, NJ.
2. Goromaru, T.; Matsuki, K.; Matsuki, Y. Gas chromatographic-mass spectrometric determination of ^{13}C-glucose level for evaluating the effect of alpha-glucosidase inhibitor acarbose on the digestion of starch in rat. Biol. Pharm. Bull. **1994**, *17* (1), 156–159.

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