ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
25 (2001) 39–52

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Determination of captopril in pharmaceutical tablets by anion-exchange HPLC using indirect photometric detection; a study in systematic method development☆

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Received 25 April 2000; received in revised form 11 September 2000; accepted 11 September 2000

Abstract

The development and validation of a significantly cost effective and simpler anion-exchange high performance liquid chromatorgaphic (HPLC) procedure than the compendial methods for the analysis of captopril in tablet dosage forms using indirect photometric detection is described. A low capacity anion-exchange column was used with potassium phthalate as the mobile phase marker and indirect detection at 280 nm. The chromatographic conditions were optimized using the Box and Behnken factorial experimental design. The method was precise and accurate with percent recovery ($\pm$%R.S.D.) of 99.8 $\pm$ 0.7% ($n = 12$) with the spiked concentrations ranging between $\pm$30% of the assay level. Youden and Steiner's robustness test, involving seven chosen variables, showed that the method is robust. Using the developed method, commercially available captopril tablets were assayed and the results were found to be comparable with those obtained by the compendial method.

Keywords: Captopril; Indirect photometric detection; Ion-exchange chromatography; Pharmaceutical tablets

1. Introduction

Captopril belongs to the group of anti-hypertensive drugs that affect the renin-angiotensin system and are commonly referred to as ACE inhibitors. The chemical name of captopril is (*S*)-1-(3-Mercapto-2-methyl-L-oxo-propyl)-L-proline. Captopril is marketed under the brand name of Capoten® by Bristol-Meyers Squibb in tablet dosage form of 12.5, 25, 50 and 100 mg and is also available from several generic manufacturers.

☆ Presented at the 1997 Annual Meeting of the American Association of Pharmaceutical Scientists, Boston, MA, November 1997. This work is part of the Ph.D. dissertation of TM. This work does not reflect the official point of view of the United States Pharmacopeia.

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0731-7085/01/$ - see front matter
PII: S0731-7085(00)00462-3

Captopril is characterized by the lack of a strong chromophore and, therefore, not able to absorb at the more useful UV–Vis region of the spectrum. For this reason, a vast number of literature articles have described derivatization techniques in which a chromophore or a fluorophore is introduced to render this UV-transparent molecule detectable at higher UV-wavelengths [1–5].

The methods included in the captopril monograph of the current USP involve the determination of captopril and it's degradation product, captopril disulfide by reversed-phase HPLC with photometric detection using a low wavelength of 220 nm, at which there is a high probability of interference from contaminants and excipients [6]. The impurity, 3-mercapto-2-methylpropanoic acid (MMA), is determined by gas chromatography following a tedious derivatization procedure using hazardous reagents.

The main objective of this study was to develop and validate an anion-exchange high performance liquid chromatographic (HPLC) procedure using indirect photometric detection for the simultaneous assay of captopril and related products in pharmaceutical tablet dosage forms, thereby offering significant cost-savings. These methods are proposed as suitable alternatives to the tedious methods described in the USP monograph for captopril. Additional specificity is achieved by using detection at higher wavelengths, thereby eliminating potential interference.

With indirect photometric detection, a detecting agent (marker) that strongly absorbs in the UV–Vis region is added to the mobile phase. Initially, the marker species are in equilibrium with sites on the stationary phase and this equilibrium is reflected by a constant absorbance value at the detector and by a high baseline. When the UV-transparent sample ions enter the detector, the concentration of marker ions decreases, resulting in a corresponding decrease in the absorbance. This decrease in absorbance is proportional to the concentration of the eluting species and is represented by a trough (negative peak) in the baseline. The detector response is an indirect one because it results from the absence of mobile phase marker rather than the presence of the analyte. Indirect photometric detection does not require additional or specialized equipment as it utilizes conventional photometric detectors and HPLC instrumentation readily available in most analytical laboratories.

2. Experimental

2.1. Apparatus

The liquid chromatograph consisted of an Hitachi Model 655A-40 or Model L-7200 autosampler (Hitachi Instruments Inc., Naperville, IL) capable of making injections of up to 100 μl in volume and an ABI Spectraflow Model 757 variable wavelength detector (Kratos Analytical, Ramsey, NJ). The chromatographic system was equipped with either Hitachi Model L-6000 or Model L-7100 piston reciprocating pump. The chromatographic data was acquired and analyzed using PeakPro™ Revision 2.2 (Beckman Instruments, Inc., Allendale, NJ) data acquisition system. For the optimization of chromatographic conditions, ECHIP™ software program (ECHIP, Inc., Hockessin, DE) was used.

2.2. Chemicals

Potassium hydrogen phthalate [KHP], *m*-nitrobenzoic acid (Fisher Scientific), citric acid monohydrate (Merck and Co., Rahaway, NJ), succinic acid, captopril and monosodium glutamate (Sigma Chemical Co., St. Louis, MO), captopril related substances [captopril disulfide (CS2) and 3-mercapto-2-methylpropanoic acid 1,2-diphenylethyl-amine salt (MMA)] were purchased from The United States Pharmacopeial Convention, Inc., Rockville, MD. All the other reagents were of analytical grade and were used as received. All the solutions were prepared with distilled and de-ionized water obtained from Milli-Q system (Millipore, Bradford, MA).

2.3. HPLC conditions

Low exchange-capacity Vydac® (Vydac, The Separation Group, Hesperia, CA) 4.6 × 250 mm, 10-μm particle size, anion-exchange analytical

columns of different lot numbers were used at a temperature of 45°C with a mobile phase consisting of 0.1 M potassium hydrogen phthalate buffer/methanol/water, 25/150/825 (v/v/v), with the pH adjusted to 6.0 with 0.1 M NaOH, and pumped at 1.0 ml min^{-1}. The mobile phase was filtered through a 0.45 μm nylon membrane filter and degassed under vacuum before use. The eluates were monitored at 280 nm. The eluting analytes were recorded as positive peaks by reversing the input polarity on the detector.

2.4. Standard stock solutions

2.4.1. Captopril and succinic acid stock solutions

An aqueous stock solution of captopril was prepared by accurately weighing a proportionate amount of captopril to give a concentration of about 5 mg ml^{-1}. A stock internal standard solution of succinic acid (SA) was prepared by dissolving in water an appropriate weight of SA to give a concentration of 1.8 mg ml^{-1}.

2.4.2. Preparation of placebo tablet matrix

A placebo tablet matrix was formulated to simulate the branded and generic tablet dosage forms without containing captopril as active ingredient. The composition of this formulation is presented in Table 1 and was used in recovery and specificity studies.

2.4.3. Standard solution

A standard solution of captopril was prepared by accurately weighing 50 mg of captopril into a 50-ml volumetric flask, pipetting a 10-ml aliquot of the stock SA solution and diluting to volume with water. The concentration of captopril and SA in this solution was 1.0 and 0.36 mg ml^{-1}, respectively.

Table 1
Placebo matrix composition for recovery studies

Component	Percent	Grams
Microcrystalline cellulose NF(avicel pH 102)	35.0	7.0
Lactose monohydrate NF	27.5	5.5
Pregelatinized starch (starch 1500)	5.0	1.0
Starch	5.0	1.0
Stearic acid	0.025	0.05
D and C yellow # 10 lake	0.01	0.02

2.5. Sample preparations

2.5.1. For recovery studies

Three different aliquots of the captopril stock solution of 7.0, 10.0 and 13.0-ml were pipetted into separate 50-ml volumetric flasks (two flasks at each level), each containing 190 mg of the tablet placebo matrix. Into each flask, a 10.0-ml aliquot of the SA stock solution was pipetted, diluted to volume with water and sonicated for ten minutes to give spiking solutions at 70, 100 and 130% of the assay level.

2.5.2. For commercial products

Commercially available tablets from two different manufacturers labeled as Product A and B were assayed by this proposed anion-exchange HPLC method. Twenty tablets of each product were ground in a mortar and an aliquot of the powder equivalent to 50 mg captopril was transferred into 50-ml volumetric flasks, 10.0-ml aliquot of the SA stock solution pipetted into each flask, diluted to volume with water and sonicated to give a final concentration of 1.0 mg ml^{-1} of captopril.

2.6. Chromatographic procedure

Exactly 25 μl of the sample solution and the standard solution were injected separately onto the analytical column by using the autoinjector equipped with a sample loop and chromatographed under the operating conditions described in previous sections. Quantitation was accomplished by comparing the peak area ratios (captopril peak area/SA peak area) of the sample to that of the standard.

2.7. Optimization of the chromatographic conditions

In order to optimize the chromatographic conditions, a three level Box and Behnken factorial design was used [7]. The concentration of KHP, %

Table 2
Concentration levels of three variables for the optimization studies

	Level	−1	0	+1
A	MM KHP	1	1.8	2.5
B	% Methanol	15	20	25
C	Column temperature (°C)	40	45	50

Table 3
Three level factorial design used in the optimization studies

A	B	C
±1	±1	0
±1	0	±1
0	±1	±1
0	0	0
0	0	0
0	0	0

organic modifier and column temperature were identified as the critical variables for the factorial design. The three levels of these variables were chosen from the results of the initial experimentation. The factorial design and the concentration range of each variable are presented in Tables 2 and 3, respectively.

Using the experimental design presented in Table 3, a set of 15 experiments was conducted by making duplicate injections of the standard solution onto the column. The average resolution, tailing factor, theoretical plates and capacity factors were measured from the resulting chromatograms. The data were analyzed by polynomial regression analysis using ECHIP™ Version 6.1 software program to obtain two- and three-dimensional plots from which the optimum conditions were determined.

2.8. Determination of linearity

Ten standard solutions containing the internal standard (SA) were prepared by diluting the appropriate aliquots of the captopril and SA stock solutions. The concentration of captopril in these solutions ranged between 20 and 200% of the assay concentration of 1.0 mg ml^{-1}. Each of the solutions was analyzed using the optimized chromatographic conditions on two different days and the peak area ratios were plotted against the amount of captopril injected.

2.9. Determination of limit of quantitation (LOQ)

A set of four dilute solutions containing captopril, SA, captopril disulfide and MMA were prepared and seven replicate injections of each solution ($n = 7$) were made. The amount of captopril injected representing the four dilute solutions was 1.2, 1.7, 2.3 and 2.9 μg, respectively. The amounts of CS2 and MMA injected were 1.0, 1.5, 2.0 and 2.5 μg, respectively. The limit of quantitation of each component was determined from the standard deviations ($n = 7$) of the peak area ratios using the extrapolation method [8]. The y-intercept was calculated from the regression analysis of standard deviation (S.D.) of the peak area ratios versus the concentration for each component. The y-intercept, representing the uncertainty of blank, was multiplied by a factor of ten to give the limit of quantitation in terms of peak area ratio. The corresponding concentration that would result in the calculated peak area was determined from the response of captopril solution of a known concentration. This calculated concentration was the limit of quantitation.

2.10. Test for robustness

The mobile phase related factors including pH, flow rate, marker concentration, % organic modifier, along with the column temperature, detector wavelength and column age were carefully chosen as the seven variables for Youden and Steiner's robustness test [9]. The effect of each variable was investigated at two levels as indicated by the upper and lower case letters in Table 4. Two different levels of spiked placebo solutions containing SA were prepared, giving 0.7 and 1.3 mg ml^{-1} of captopril. The recovery of these solutions was determined following Youden and Steiner's experimental design presented in Table 5. A total of eight experiments were conducted in order to identify variables that may have to be controlled in order to obtain accurate assay results.

3. Results and discussions

3.1. Development of the analytical method

The elution of captopril was initially carried out using a 4.6×250 mm, 5 μm Supelcosil LC-SAX column containing stationary phase comprised of silica particles with quaternary aminopropyl groups as the bonded-phase. *m*-Nitrobenzoic acid ($pK_a = 3.5$) at 2 mM concentration was investigated as the eluent marker. The pH of the mobile phase was adjusted to 6.8 to ensure that the marker was in the ionized form and the detector was set at 254 nm. The background absorbance at 310 nm was approximately 0.3 au.

A 50-μl injection of a captopril solution prepared in water resulted in a very broad negative peak at a retention time of 13 min. A positive peak immediately followed by a negative peak was observed at the void volume. A late eluting positive peak was observed at about 45 min. *The detector polarities were reversed so that the negative peaks were registered as positive*. The large positive peak at the retention time of 13 min was investigated by injecting solutions containing different concentrations of captopril. The magnitude of this negative peak changed proportionally with the concentration of captopril, thus indicating that the negative peak was caused by displacement of the eluent from the stationary phase by captopril. The late eluting positive peak was identified as the displaced eluent appearing at the characteristic retention time of *m*-nitrobenzoic acid. Subsequent replicate injections of captopril resulted in rapid deterioration of the efficiency of the column. The retention time of captopril decreased gradually over time and eventually captopril eluted at the void volume. From these studies, it became evident that this column was a high exchange-capacity column and that the exchange sites had strong affinity for the eluent. The exchange sites were gradually occupied by the eluent

Table 4
Variables and their levels for robustness test studies

Selected variable	Units	Abbreviation[a]	High level	Low level
pH	–	A,a	6.1	5.9
Flow	ml min^{-1}	B,b	1.1	0.9
KHP concentration	MM	C,c	2.7	2.3
% Organic	%	D,d	16	14
Column temperature	°C	E,e	47	43
Wavelength	nm	F,f	282	278
Column	–	G,g	New	Old

[a] Upper case letter represent high level, lowercase letter represents low-level value of the variable.

Table 5
Youden and Steiner's robustness test experimental design

	Experiment #							
Variables	1	2	3	4	5	6	7	8
pH	A	A	A	A	a	a	A	a
Flow (ml min^{-1})	B	B	b	b	B	B	B	b
MM KHP	C	C	C	c	C	c	C	c
% Organic	D	D	d	d	d	d	D	D
Column temperature (°C)	E	E	E	e	e	E	e	E
Wavelength	F	F	f	F	F	f	f	F
Column	G	G	g	G	g	G	G	g
Observed result	s	t	u	v	w	x	y	z

molecules leading to decreased column efficiency. The column was regenerated by flushing it with a mixture of dilute nitric acid and methanol. The experiments were repeated on the 'regenerated' column. The same observations were made as before and the column efficiency again decreased with time.

In the subsequent studies, all the chromatographic conditions were kept the same as before, except that *m*-nitrobenzoic acid was replaced with potassium hydrogen phthalate (KHP) as the eluent and detector set at 280 nm. A set of five captopril standard solutions ranging in concentration from 0.08 to 1.05 mg ml^{-1} was injected. The linear regression analysis of the peak area of captopril versus captopril concentration gave a correlation coefficient of 0.9999, indicating that the detector response was linear. The injection repeatability was acceptable with the R.S.D. of the peak areas of a captopril being 1.5% ($n = 5$). These results could not be replicated using the same column. As observed before, the column efficiency decreased with time, again indicating that the column was a high-capacity column.

At this point, it was decided to search for a low capacity anion-exchange column. A literature search revealed that a low capacity anion-exchange column available from Vydac was previously used for the detection of anions by ion-chromatography [10–12]. This column is comprised of a silica-based material with large pores containing low capacity anion exchange (quaternary amine) groups. KHP was chosen as the eluent for this column although earlier experiments had demonstrated that both m-nitrobenzoic acid and KHP were suitable UV-absorbing eluents for the indirect detection of captopril. This decision was based on the fact that KHP was proven to be a successful eluent in indirect photometric detection [13]. The mobile phase was comprised of 0.8 mM KHP with a pH of 6.5. A positive captopril peak exhibiting extreme fronting was observed at a retention time of approximately 13 min. In order to minimize the fronting of the peak, the effect of the type of sample preparation solvent, the type and percent of organic modifier in the mobile phase, the pH of the mobile phase and the flow rate were investigated. The addition of the organic modifier, methanol, to the mobile phase had a significant effect on the peak shape. This information indicated that the separation process also involved some reversed-phase mechanism. Replacing methanol with the appropriate volume of acetonitrile to give equivalent polarity did not have much effect on the peak shape. The tailing factor was still less than 0.80 but the peak area response was linear ($r > 0.999$) and reproducible (R.S.D. $< 2\%$, $n = 5$). The mobile phase that provided the best peak shape was comprised of 10% acetonitrile in water containing 1.0 mM KHP with the pH adjusted to 6.5 with 1 N solution of KOH.

The choice of an appropriate internal standard was made by injecting solutions of citric acid, ammonium formate, potassium acetate, propanoic acid, hexanoic acid and monosodium glutamate. Monosodium glutamate (MSG) was chosen as the internal standard. It appeared as a negative peak prior to the captopril peak with a baseline resolution of greater than 2.0. A chromatogram obtained by using the initial chromatographic conditions is presented in Fig. 1. Both peaks in the chromatogram still exhibited significant fronting. The next factor that was manipulated in order to improve peak shape was the column temperature. An increase in column temperature resulted in significant improvement in the efficiency of both the peaks, especially in the captopril peak. The theoretical plate number, *N*, for captopril at ambient temperature was 682 and it increased significantly to 939 at 60°C. The improvement in efficiency at higher temperatures can be attributed to the lower diffusion coefficients resulting from lower viscosity of the mobile phase. The increase in the column temperature only reduced the bandwidth but did not improve the peak shapes. The chromatograms obtained at ambient and 60°C are shown Fig. 2. However, the column started exhibiting signs of inter-particular void spaces at 60°C. Therefore, the column temperature was reduced to 50°C.

The eluent (KHP) concentration was increased to 2 mM in order to investigate if it would improve the peak shapes and the efficiency. This resulted in the most significant improvement in the peak shapes and efficiencies. The tailing fac-

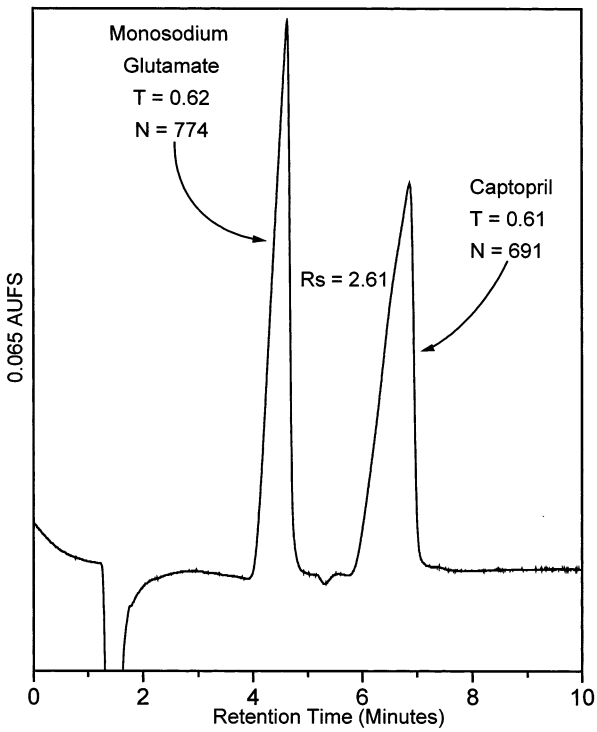

Fig. 1. Liquid chromatogram using the initial chromatographic conditions (detector polarity reversed).

tors and efficiencies (theoretical plates) for MSG and captopril improved to 0.71 and 0.89, and 1263 and 2427, respectively.

In order to reduce the background absorbance without changing ionic strength, the KHP concentration was reduced to 1.0 mM while 1.0 mM potassium chloride, a non-UV absorbing salt was added to the mobile phase. Under these conditions, the background absorbance decreased to 0.55 AU and a positive peak appeared in between the two peaks of interest. After about 15 replicate injections of the standard solution containing MSG and captopril, the column efficiency deteriorated and the resolution between the positive peak and peaks of interest reduced significantly. Ultimately the positive peak co-eluted with the captopril peak. It was concluded from these studies that the critical factors affecting the elution of captopril were ionic strength of the mobile phase, percent organic modifier in the mobile phase and the column temperature. The column temperature and the percent organic in the mobile phase affected the peak shapes and efficiency. The ionic-strength of the mobile phase affected the efficiency and the capacity factors. Controlling the retention times, especially that of the system peak is of extreme importance in indirect photometric detection. Since the system peak elutes late in the chromatogram, the run times have to be very long; hence every attempt must be made in order to reduce its retention time so that the run times can be reduced to a reasonable duration. The most convenient way of reducing the retention time of the system peak is by increasing the ionic strength of the mobile phase. Unfortunately this also reduces the retention times of the peaks of interest, which in turn affect the specificity of the system.

The ionic strength as discussed earlier in this section can be increased by either increasing the eluent concentration or by adding a non-UV absorbing salt to the mobile phase. Both the options have their own undesirable effects and must be thoroughly evaluated. The latter approach results in undesirable extraneous peaks in the chro-

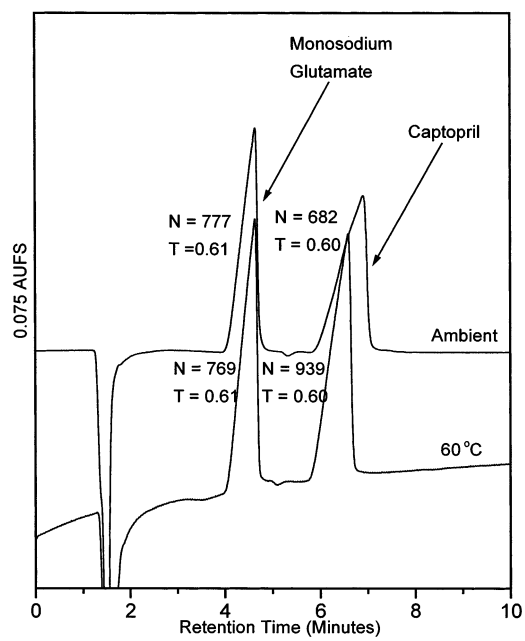

Fig. 2. Liquid chromatograms showing the effect of temperature on column efficiency.

Table 6
ECHIP regression analysis results[a,b]

	N	*k'*	*T*	*Rs*
Marker concentration (mM)	***	***	***	***
% Organic		*		*
Temp (°C)	*			
Marker concentration × % Organic			*	
Marker concentration × temperature				
% Organic × temperature				
Marker concentration[b]		***		***
% Organic[b]		*		*
Temperature[b]				

[a] Based on ECHIP optimization software program.
[b] *N*, number of theoretical plates, *k'*, capacity factor, *T*, tailing factor, and *Rs*, resolution.

matograms and the former option results in high background absorbance and reduced sensitivity. Sensitivity is not a major concern in the analysis of captopril because the assay concentration is fairly high. Therefore, increasing the eluent (marker) concentration in this case is a more viable option.

3.2. Optimization of the chromatographic procedure

The average theoretical plates, tailing factor, capacity factor and resolution for the two peaks of interest were calculated from the chromatograms obtained using the 15 experimental conditions on the basis of the Box and Behnken experimental design. The ECHIP optimization software program analyzed these data in order to determine optimum chromatographic conditions.

The results demonstrate that the experimental conditions had a similar effect on both peaks which means that optimization of the chromatographic conditions for one peak would result in optimized conditions for the other peak as well. The captopril peak was chosen for this purpose and subsequent efforts were concentrated in optimizing chromatographic conditions for the captopril peak only. The results indicate that the conditions in Experiment 2 of 2.5 mM KHP, 15% organic modifier and 45°C resulted in the highest resolution and average theoretical plates and best tailing factor for both peaks. Even without any statistical treatment of the data, these conditions seemed to give the optimum chromatographic results. This observation was confirmed by data analysis by using the ECHIP optimization program.

The ECHIP software analyzes the data by regression analysis. Based on the quadratic relationships and by ANOVA analysis, the program then calculates the significance of each variable on the chromatographic parameters. A simple summary of the regression analysis is presented in Table 6. The effect of each variable is represented by an 'asterisk.' The more the number of asterisks, the higher the influence the variable has on the given chromatographic parameter. The data shows that the marker (KHP) concentration has the largest influence on all the four chromatographic parameters. This is followed by the percent organic content as it has influence on two parameters. The least important variable is the column temperature, which effects only one parameter. In the subsequent data analyses, the optimum KHP concentration and % organic content were also determined from the surface contour plots and 3-dimentional plots. A 3-dimentional plot and a surface contour plot showing the effect of KHP concentration and % organic content on the resolution is presented in Figs. 3 and 4, respectively.

Later studies revealed that the impurity, MMA peak co-eluted with the internal standard (MSG) peak making it necessary to replace MSG with a different internal standard that would elute later than captopril and away from the void volume. Using the optimized chromatographic conditions, several non-UV absorbing di- and tricarboxylic acids were injected into the column. This decision was based on the rationale that the ionized multifunctional carboxylic groups should have higher affinity for the ion-exchange sites and hence elute later than a molecule such as captopril, which has only one carboxylic acid group. Succinic acid (SA) was selected as the internal standard. By using the optimized chromatographic conditions, the succinic acid peak eluted later than captopril and

was very symmetrical. There was adequate resolution between captopril and SA. Subsequent studies indicated that the optimum conditions obtained for MSG are valid for SA. A chromatogram showing captopril and SA obtained using the optimized conditions is presented in Fig. 5.

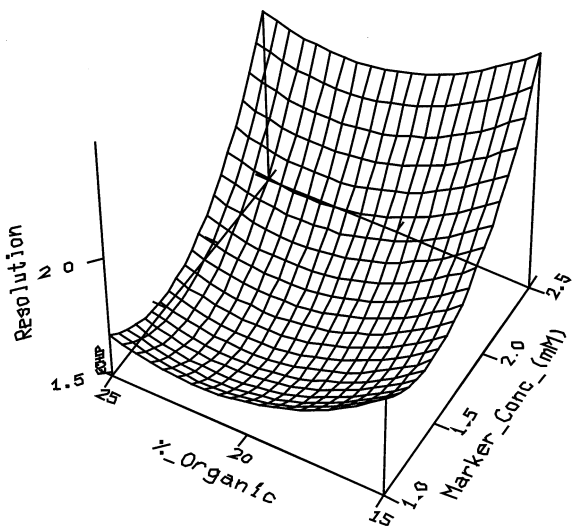

Fig. 3. 3-Dimensional plot showing the effect of % organic and marker concentration on resolution (Off axis = 45°C).

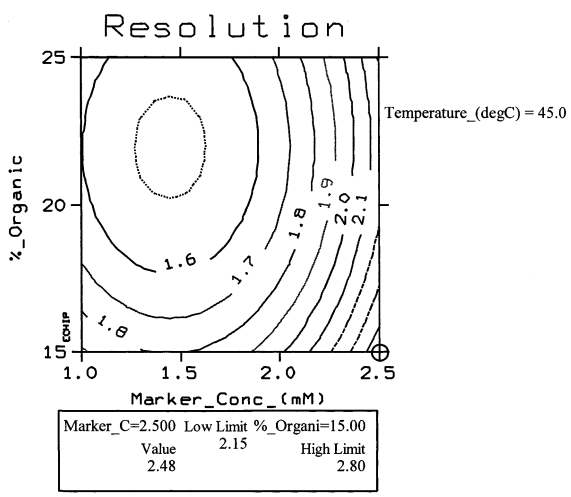

Fig. 4. Contour plot showing the optimum chromatographic conditions required to obtain highest resolution (Temperature = 45°C).

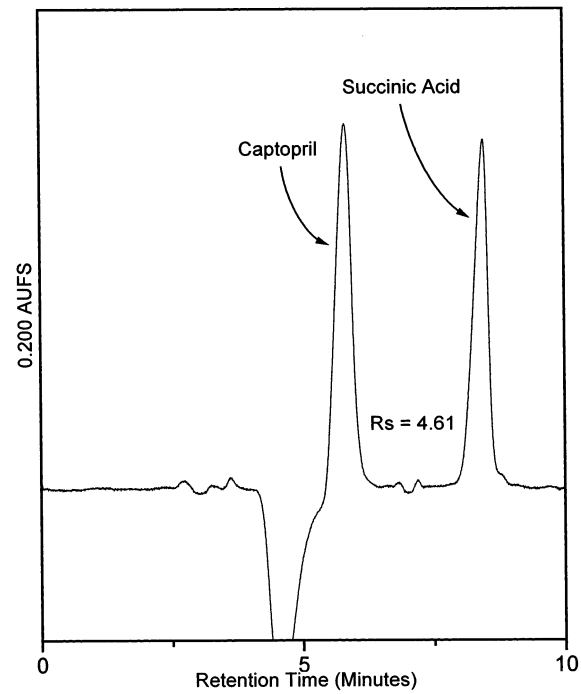

Fig. 5. Liquid chromatogram of captopril and succinic acid standard solution at the optimized chromatographic conditions

3.3. Validation of the method

The validation of the method was carried out by determining the accuracy, precision, linearity, specificity, limit of quantitation and robustness of the method. The accuracy of the method was determined by assaying the spiked tablet placebo matrix solutions of known concentration and then the percent recoveries of captopril were determined at three levels corresponding to 70, 100 and 130% of the assay level of 50 mg (1.0 mg ml^{-1}) and are presented in Table 7. The overall percent recovery ($\pm$%R.S.D.) was $99.82 \pm 0.91\%$ ($n = 12$). Analysis of variance (ANOVA) was done to determine if there was any difference in the percent recovery at the three different levels. The results indicated that there was no difference at 95% confidence level as the calculated F value of 0.9896 was lower than the tabulated value of 4.256 using 2.9° of freedom. It can be concluded that there was no bias involved in the results due

Table 7
Percent captopril recovery from tablet placebo matrix

Captopril added (mg)	% Recovery	Average	S.D.	% R.S.D. ($n=4$)
35.27 (70%)[a]	99.02			
	99.75			
	98.30			
	99.78	99.21%	±0.70	0.71
50.39 (100%)[a]	100.80			
	101.68			
	99.47			
	98.76	100.18%	±1.31	1.31
65.51 (130%)[a]	100.07			
	99.71			
	100.44			
	100.10	100.08%	±0.30	0.30
Overall ($n=12$)	99.82%	±0.91	0.91%	

[a] = % of assay level of 50 mg.

to the excipients at ±30% of the assay level as the mean% recovery at each level was the same. It is also concluded that it is acceptable to calculate the overall percent recovery by taking the average of all 12 observations.

Assay repeatability of the analytical procedure expressed as % R.S.D. was determined from the data and is shown in Table 7. The overall % R.S.D. of the assay ($n=12$) was less than 1.0% and the R.S.D. of assay results ($n=4$) at each level was less than 2.0%. Injection repeatability was determined by making replicate injections ($n=6$) of a standard solution corresponding to the assay concentration of 1.0 mg ml^{-1} on three different days with at least 3 days elapsing in between. The peak area (PAR) and peak height ratio (PHR) was calculated from each chromatogram. The % R.S.D. ($n=6$) of the PHR on all the 3 days was consistently higher than the %R.S.D. of PAR. and was also greater than the desired precision of 2.0. Therefore, it was decided to use PAR in this assay procedure.

The linearity of the method was determined by regressing the average peak area ratio obtained versus the amount of captopril injected. A linear response was obtained on two different days over the concentration range of 5–50 μg captopril injected. A typical regression equation of $A = 0.03850C - 0.02350$ ($r=0.9990$) was obtained (A, peak area ratio, C, μg captopril injected). Specificity of the method was measured from the response of captopril in the presence its degradation products, precursors, excipients and known impurities. The components were injected individually in order to identify peaks. A chromatogram containing captopril and related compounds captopril disulfide and MMA obtained by using the method is presented in Fig. 6. The chromatogram demonstrates that the method is 'stability indicating' as the resolution between all the peaks was greater than 1.5, except for the resolution between captopril and MMA peaks, in which case it was 1.1.

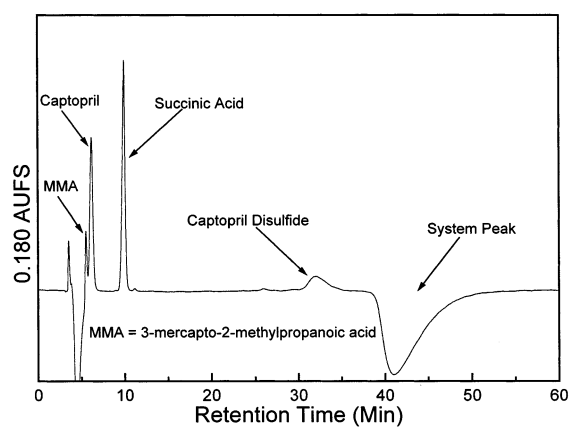

Fig. 6. Liquid chromatogram of a solution containing a mixture of captopril, succinic acid, captopril disulfide, and 3-mercapto-2-methylpropanoic acid

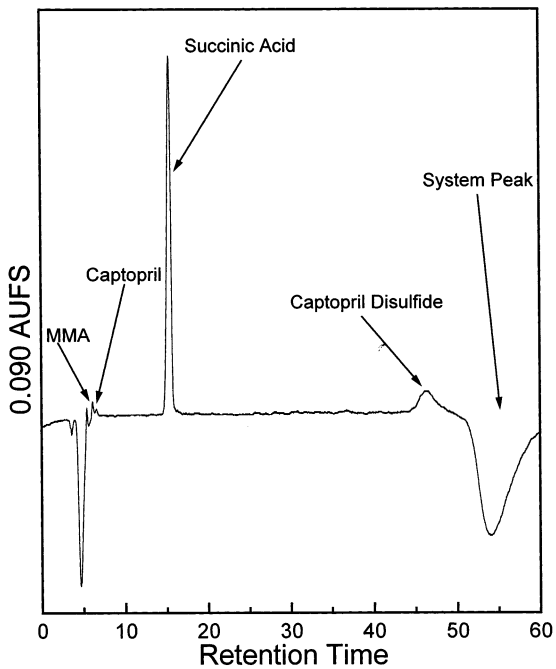

Fig. 7. Liquid chromatogram of a solution containing captopril, 3-mercapto-2-methylpropanoic acid (MMA), and captopril disulfide at LOQ concentrations.

Specificity was also determined by analyzing an extract obtained by sonicating the tablet placebo matrix in water. There were no extraneous peaks in the chromatogram that indicated coelution with the peaks of interest.

Extrapolation method was utilized for determination of limit of quantitation (LOQ) of captopril, MMA (impurity) and CS2 (degradation product). Four dilute samples containing captopril, MMA, CS2 and succinic acid were prepared. Each sample was analyzed seven times using the proposed chromatographic conditions and the standard deviation of the peak area ratio ($n = 7$) was determined. The LOQ was then calculated using the following equation:

$$\mathrm{LOQ} = \frac{\mathrm{Cst} \times 10\, S_0}{\mathrm{Ast}}$$

where, Cst, concentration of the standard solution; S_0, standard deviation at concentration of zero, equal to the y-intercept of regression line, and Ast, peak area ratio of the standard.

The LOQ for captopril, MMA and CS2 were determined to be 0.70, 1.78 and 3.82 ng injected, respectively. A chromatogram obtained by injecting a solution containing the three components at the LOQ concentrations is presented in Fig. 7. The desired LOQ levels for MMA and CS2 at 0.1 and 0.2%, respectively, of captopril at the assay level of 25 μg is 31.3 and 92.6 ng, respectively. Therefore, the proposed method exceeds the LOQ requirement for MMA and CS2.

Robustness of an analytical procedure is measured in order to determine its capacity to remain unaffected by small and deliberate variations in the method parameters. It is an indicator of the reliability of the analytical procedure during normal usage. The experimental design proposed by Youden and Steiner was used and eight experiments were conducted in order to determine the effect of seven different variables. The variables and the experimental design are presented in Tables 4 and 5. The observed results to be evaluated were the recoveries of captopril from the tablet placebo matrix at two concentration levels of 0.7 and 1.3 mg ml^{-1}. The results were designated by letters s through z.

From the results of these eight experiments, the effect of each variable can be determined. For example, to find the effect of mobile phase pH, the averages of $(s+t+u+v)/4$ and $(w+x+y+z)/4$ are compared. The two groups of experiments with four determinations of pH at low and high values are set up in such a way that each group contains the other six factors twice at high and twice at low levels. The effect of all the other six factors cancel out only leaving the effect of changing the pH from A to a. The results of this example are tabulated in Table 8. The differences obtained between the two levels of each variable are ranked in decreasing order in Table 9.

The results from Table 10 indicate that the mobile phase pH is the most important variable that needs to be controlled. If pH is carefully controlled and not considered as a variable, the standard deviation (S.D.) ($s^2 = 2/7\Sigma d^2$) drops to 0.566 from 0.797 for the percent recovery at the low level of 35 mg. This decrease represents a

21% drop in the standard deviation. The mobile phase pH does not impact the recoveries at the high level of 65 mg. These results indicate that the method is fairly robust and can withstand minor fluctuations in the operating variables that may occur in the routine usage of the method. The linearity of the method was not affected by small fluctuations. The correlation coefficient was better than 0.999 under all the experimental conditions.

3.4. System suitability test

System suitability of the proposed method was established in order to ascertain that the chromatographic method has adequate precision and resolution prior to its use. Twenty chromatograms were randomly chosen and the average and S.D. for resolution between captopril and succinic acid, retention times, tailing factors and efficiency of

Table 8
Effect of mobile phase pH on captopril recovery and linearity

% Recovery of Captopril				
		Low Level	High Level	Std. Curve.
pH	Experiment	35 mg[a]	65 mg[b]	Correlation coefficient (r)
	1	98.4	99.3	0.9998
5.9	2	98.8	98.6	0.9999
	3	99.1	100.0	0.9999
	4	98.2	101.2	0.9997
	Average	98.6	99.8	0.9998
	S.D.	0.4	1.1	
	5	98.3	99.1	0.9999
6.1	6	98.9	100.1	0.9996
	7	100.5	100.2	0.9999
	8	100.4	100.1	0.9999
	Average	99.5	99.9	0.9998
	S.D.	1.1	0.5	
	Differences	0.9	0.1	0.0000

[a] Corresponds to 0.7 mg ml^{-1} in final test solution.
[b] Corresponds to 1.3 mg ml^{-1} in final test solution.

Table 9
Robustness test results

	Difference (d) in		
	% Recovery of captopril		
Variable	At 0.7 mg ml^{-1}	At 1.3 mg ml^{-1}	Standard curve correlation coefficient (r)
Mobile Phase pH	0.9	0.1	0.0000
Column Age	0.2	0.7	0.0001
Marker concentration (mM)	0.0	0.5	0.0001
% Organic modifier	0.9	0.6	0.0001
Wavelength (nm)	0.3	0.1	0.0001
Mobile phase flow (ml min^{-1})	1.0	1.1	0.0001
Column temperature (°C)	0.2	0.1	0.0001
Standard deviation[a]	0.797	0.697	–

[a] Calculated using the formula: $s^2 = 2/7\ \Sigma d^2$.

Table 10
Assay results obtained by the USP and the proposed anion-exchange indirect photometric detection (IPD) method

Tablet	Mean tablet assay value (mg)			
Label	Product A		Product B	
Claim (mg)	USP method[a]	IPD method[b]	USP method[a]	IPD method[b]
12.5	12.3 ± 0.1	12.6 ± 0.2	12.2 ± 0.03	12.3 ± 0.1
25	23.4 ± 0.2	23.8 ± 0.2	24.5 ± 0.1	24.4 ± 0.3
50	50.0 ± 0.1	50.0 ± 0.5	48.9 ± 0.2	49.9 ± 0.4
100	N/A	N/A	99.4 ± 0.4	99.6 ± 0.5

[a] Duplicate assays.
[b] Triplicate assays; N/A, not available.

the two peaks were calculated. A close analysis of the data obtained during the validation studies revealed that the retention times of captopril and succinic acid peaks decreased with the number of injections made on the column. The retention time of succinic acid in particular was affected by increased usage of the column and ranged from 8.91 to 16.39. The retention time of captopril was not effected as much and it ranged between 5.59 and 6.09. The decrease in the retention time coincided with a corresponding decrease in the efficiency (N) and resolution between captopril and succinic acid. Consequently, the resolution had a very wide range of 12.46–4.37. A resolution of 1.5 is considered as baseline resolution. Therefore, even the lowest observed resolution of 4.37 was acceptable. The average tailing factors of captopril and succinic acid were acceptable with values of 1.08 ± 0.23 and 0.89 ± 0.20, respectively.

The injection repeatability, expressed as % R.S.D., was determined by replicate injections ($n = 6$) of a standard solution on different days was $< 1.5\%$. This is well below the USP repeatability requirement of 2%. On the basis of this data, it is recommended that for the system to be suitable for use, the R.S.D. ($n = 6$) of the peak area ratios should not be greater than 2.0%.

3.5. Comparisons with USP methods: assay of commercial products

Different strengths of commercially available tablets from two different manufacturers, labeled as Product A and B, were assayed by the proposed HPLC method and the method included in the current USP monograph. The results obtained from the two methods are compared in Table 10 and a plot of the results for Product A is presented in Fig. 8. Good agreement was observed between the two methods of analysis. A correlation coefficient of 0.9999 was obtained for both

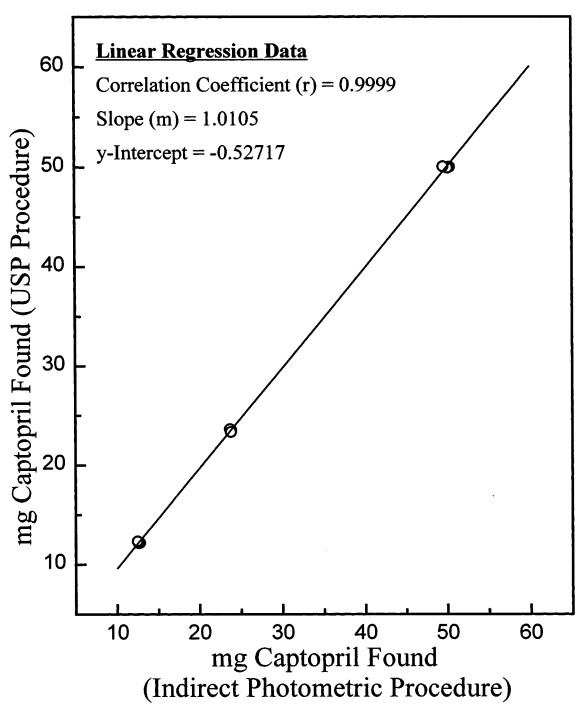

Fig. 8. Comparison of the assay results of Product A by the proposed anion-exchange (IPD) method and the USP method.

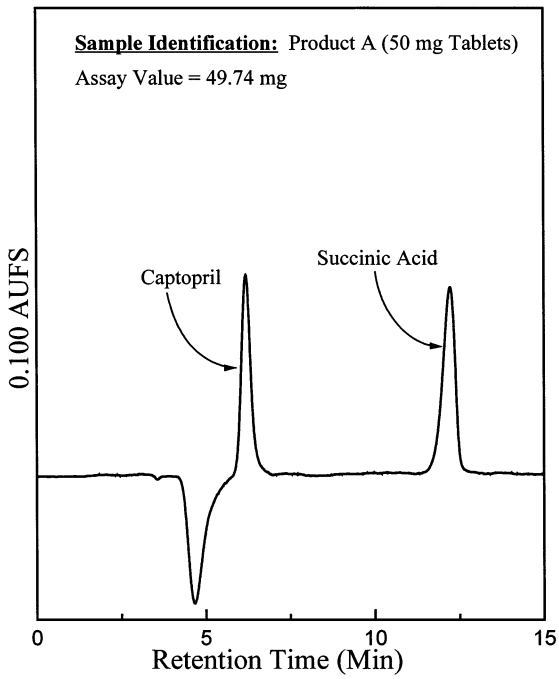

Fig. 9. Liquid chromatogram of Product A under the proposed experimental conditions.

Product A and Product B when the two assay values were plotted against each other. This indicates that the proposed method has the potential to be used as good alternative to methods for the assay of captopril included in the USP monograph. A typical chromatogram obtained using the proposed method for the assay of Product A is presented in Fig. 9.

4. Conclusions

The proposed anion-exchange HPLC method using indirect photometric detection is simpler and cost-effective than the current compendial procedures. Using this method captopril and related products can be analyzed simultaneously as opposed to the use of two tedious methods included in the USP monograph. The results indicate that the proposed method is precise, accurate, robust, specific and sensitive for the analysis of captopril and related products in commercially available tablet dosage forms.

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