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PENTAZOCINE TABLET ANALYSIS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A rapid extraction ion-pair reversed-phase system has been demonstrated for analysis of pentazocine hydrochloride in tablets. This high-performance liquid chromatographic method was also shown to separate other members of a series of benzomorphans. A favorable comparison was found between results by this method and a normal-phase HPLC method for pentazocine. The effects of ion-pairing agent and of concentration were investigated in view of possible retention mechanisms. A review of previous analytical methods for pentazocine is given.

INTRODUCTION

Benzomorphan analgesics have been widely studied in human and animal species following their introduction in the 1960's. Notable among these has been pentazocine, an analgesic agonist with certain degrees of antagonist activity. A variety of analytical techniques have been employed by workers mainly in the field of biological disposition and pharmacokinetics of pentazocine. Spectrofluorometric methods[1–4] were introduced early as were gas chromatographic methods. Initially flame ionization detection was used, with and without derivatization of pentazocine[5–9]. A nitrogen detector found some application[10] however more recent work has been done using the sensitive electron capture detector[11–13]. Sensitive and specific radioimmunoassay procedures have been used for analysis of pentazocine in human and dog plasma and urine[14,15].

The use of high-performance liquid chromatography (HPLC) for pharmaceutical analysis gives sensitive, selective, rapid and stability-indicating results. These considerations become important when large numbers of samples are encountered routinely. This method eliminates the need and time for derivatization, possible hazards associated with the use of radioactivity and time consuming antibody production. Pentazocine has been studied by HPLC in a series of narcotic agonists using a reversed-phase, phosphate buffer–methanol system[16]. While a normal-phase method was used to determine pentazocine absorption from aqueous suspensions[17], the results of analysis for pentazocine in one dosage form have been published[18]. This was for repackaged pentazocine hydrochloride solution. The HPLC method used was

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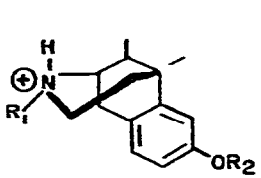
TABLE I

PENTAZOCINE ANALYTICAL METHODS

Materials analyzed: p = plasma; u = urine; b = blood; d = solution dosage form; c = cerebrospinal fluid; br = brain.

Method	*Ref.*	*Recovery (%)*	*Sensitivity*	*Linear range*	*Statistics*
Fluorometric	1 (p)		0.03 μg/ml		
	2	90	0.2 μg/ml		
	3,4 (u)(p)(c)		0.15-5 μg/ml		±10%
Gas chromatography					
F.I.D.	5,6 (br)	96		25-500 ng	
	7 (b)	98	0.2 μg/ml	20-40 ng/ml	5% S.D.
	(u)	93	1 μg/ml		3% S.D.
	8,9 (u)	97	0.5 μg/ml	0.1 30 μg/ml	4% S.D.
Nitrogen detector	10 (b)	100.3	0.119-2 μg/ml	2.4 40 ng	4.3% S.D.
Electron capture	11,12 (p)		25 ng/ml		7% C.V.
			5 ng/ml	1-50 ng/ml	5.5% C.V.
	13 (u)	91–104 (no hydrolysis)	5 ng		5-6% S.D.
		97–100 (with hydrolysis)			3-4% S.D.
	(p)	92-95	100 ng/ml		11-13% S.D.
		72–100	25 ng/ml		14-44% S.D.
Radioimmunoassay	14 (p)	91–118	5 ng/ml		
	15 (p)			1–100 ng/ml	8.2–11.6% C.V.
HPLC	18 (d)	99	0.2 mg/ml		0.0035 S.D. 1.8% C.V.



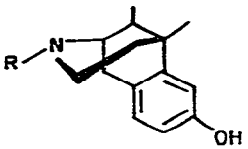
COMPOUND R_1 R_2

I $-CH_2CH=C(CH_3)_2$ $-H$

II $-CH_2$-cyclopropyl $-H$

VII $-CH_2CH=C(CH_3)_2$ $-CCH_3$ (O)

IX $-CH_2-C_6H_5$ $-H$

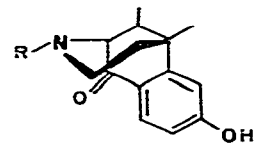


COMPOUND R

III $-CH_2CH_2C(OH)(CH_3)_2$

IV $-H$

VIII $-C$(O)-cyclopropyl



COMPOUND R

V $-CH_2$-cyclopropyl

VI $-CH_2CH=C(CH_3)_2$

Fig. 1. Structures of benzomorphans studied.

reversed-phase with a heptanesulfonic acid salt pairing ion. Table I summarizes previous analytical methods with an indication of the sensitivity, recovery and linear range obtained where available. The present study reports the application of an ion-pairing reversed-phase HPLC method for pentazocine in tablets as well as its use for other members of the benzomorphan series. In addition the reversed-phase ion-pairing retention mechanism of benzomorphans is investigated. Studies concerning the effect of size and concentration of the pairing ion on benzomorphan retention are presented.

EXPERIMENTAL

Reagents and materials

Methanol (MCB, glass dist., LC grade), chloroform (MCB, glass dist., LC grade), isopropylamine (MCB), water was house distilled and filtered (Millipore, 0.45 μm), sodium octanesulfonate, sodium heptanesulfonate, sodium hexanesulfonate, sodium pentanesulfonate, sodium decyl sulfate and sodium dodecyl sulfate (Eastman-Kodak). Benzomorphans studied (Sterling-Winthrop): I, pentazocine hydrochloride (2α,6α,11*R**)-(±)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocin-8-ol hydrochloride; II, cyclazocine hydrochloride, (2α,6α,11*R**)-(±)-3-(cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-2,6-methano-3-benzazocin-8-ol hydrochloride; III, pentazocine hydrate, (2α,6α,11*R**)-(±)-1,4,5,6-tetrahydro-8-hydroxy-α,α,6,11-tetramethyl-2,6-methano-3-benzazocine-3(2*H*)-propanol; IV, pentazocine nor-base, (2α,6α,11*R**)-(±)-

1,2,3,4,5,6-hexahydro-6,11-dimethyl-2,6-methano-3-benzazocin-8-ol; V, ketocyclazocine, (2α,6α,11*S**)-(±)-3-(cyclopropylmethyl)-3,4,5,6-tetrahydro-8-hydroxy-6,11-dimethyl-2,6-methano-3-benzazocin-1(2*H*)-one; VI, ketopentazocine, (2α,6α,11*S**)-(±)-3,4,5,6-tetrahydro-8-hydroxy-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocin-1(2*H*)-one; VII, pentazocine acetate (2α,6α,11*R**)-(±)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocin-8-ol acetate (ester) hydrochloride; VIII, Win 20722, (2α,6α,11*R**)-(±)-3-(cyclopropylcarbonyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-2,6-methano-3-benzazocin-8-ol; IX, Win 28389, (2α,6α,11*R**)-(±)-1,2,4,5,6-hexahydro-6,11-dimethyl-3-(phenylmethyl)-2,6-methano-3-benzazocin-8-ol hydrobromide.

The above names conform with current Chemical Abstracts nomenclature.

Chromatography

Reversed-phase ion-pairing. The mobile phase was prepared following a modification of a method for pentazocine lactate. 600 ml of a 0.005 *M* sodium octanesulfonate solution (1.0814 g l water) was added to a 1-l mixing cylinder, diluted to 1 l with methanol and acidified with 1.0 ml phosphoric acid. The solution obtained having a pH of 2.4 was degassed before use by ultrasonication. An Altex Model 110A pump was used at 1.5–2.0 ml min along with a μBondapak C_{18} column (Waters) (30 cm × 3.9 mm I.D.). The detector was an Altex Model 153 at 280 nm with an absorbance of 0.08 a.u.f.s. The analytical wavelength was chosen by use of a Perkin-Elmer LC-75 UV detector with stopped-flow scanning capabilities. A Micromeritics 725 Autoinjector or a Rheodyne 7125 sample injector was used along with a Varian A-25 recorder.

Standard preparation. Approximately 28 mg of previously dried pentazocine hydrochloride equivalent to 25 mg pentazocine base were weighed accurately and transferred to a 50-ml volumetric flask. This was dissolved in and diluted to the mark with mobile phase.

Sample preparation. For the reversed-phase linearity study, pentazocine hydrochloride samples equivalent to 0%, 80%, 100% and 120% of tablet content were added in duplicate sets to placebo mixtures contained in 50-ml glass-stoppered graduated cylinders. These were extracted by adding 50.0 ml mobile phase, sonicating for 1 min and shaking intermittently for 15 min. The suspension was then filtered (Whatman No. 1 paper) and 20-μl samples were injected.

For reversed-phase replicate, analysis, pentazocine hydrochloride equivalent to 100% average tablet content and placebo mixture were intimately ground using a mortar and pestle. Weights of this mixture were extracted following the above procedure.

Normal phase. The mobile phase was prepared by adding 960 ml chloroform to a 1-l mixing cylinder, diluting to 1 l with methanol, adding 2.0 ml isopropylamine and degassing before use. A Waters M6000 pump was used at 1.2 ml/min with a Partisil 5 25 5-μm silica column (25 cm × 4.6 mm I.D.) (Whatman). The detector was a Waters 440 at 280 nm with an absorbance of 0.02 a.u.f.s. A Micromeritics 725 Autoinjector was used along with a Fisher Recordall 5000 recorder at 0.1 in./min.

Normal phase replicate analysis. Samples of pentazocine hydrochloride tablet mixture equivalent to one tablet weight were accurately weighed and placed in 50-ml glass-stoppered graduated cylinders. 50.0 ml of 0.035 *N* sulphuric acid–methanol

(1:1) was added to each, shaken 15 min intermittently, sonicated 2 min and filtered (Whatman No. 1). 10.0 ml were pipetted into 125-ml separatory funnels followed by the addition of 30 ml water and 5 ml 10% Na_2CO_3 solution. These were shaken briefly and extracted by the addition of 60 ml chloroform for 1 min. These were filtered (Whatman No. 1) into 100-ml volumetric flasks and filled to the mark with chloroform. A 20-μl sample was chromatographed.

Standard preparation. Approximately 28 mg of pentazocine hydrochloride standard, accurately weighed and dried, were placed in a 100-ml volumetric flask, dissolved in and diluted to the mark with 0.035 *N* sulphuric acid–methanol (1:1). 10.0 ml were pipetted into a 125-ml separatory funnel and extracted following the above procedure.

Minimum quantifiable limit reversed-phase study. Pentazocine hydrochloride standard in mobile phase was serially diluted using mobile phase giving 1:10, 1:100 and 1:1000 dilutions. These were analysed using the most sensitive detector setting of 0.005 a.u.f.s.

Benzomorphan analog reversed-phase chromatography. Compounds I–VII and IX were dissolved in mobile phase at the 0.5 mg/ml level. Compound VIII was dissolved in mobile phase at the 0.1 mg/ml concentration. Samples of these solutions were chromatographed and capacity factors determined.

Pairing ion concentration effect. Standard solutions of pentazocine hydrochloride and cyclazocine hydrochloride (I and II) were chromatographed in the reversed-phase ion-pairing mode described above. Concentrations of octanesulfonate pairing ion ranged from 0 to 18 m*M* in the mobile phase with no additional supporting electrolyte. Runs were also made with mobile phases containing sodium chloride to maintain constant ionic strength of 18 m*M* with the octanesulfonate.

Effect of pairing ion carbon chain length. Mobile phases were prepared as described above for sodium octanesulfonate using the following pairing ions: sodium pentanesulfonate, sodium hexanesulfonate, sodium heptanesulfonate, sodium decyl sulfate and sodium dodecyl sulfate. Samples of pentazocine hydrochloride and cyclazocine hydrochloride were chromatographed and capacity factors determined.

Quantitative determinations

Calculations of pentazocine content were carried out by use of the external standard technique of bracketting samples with standards. A Hewlett-Packard 3354 computer system was used to calculate peak heights and areas. Calculations were also done by the manual peak height method. A BASIC least squares program was run on linearity samples giving slopes, *y* intercept, average percent recovery, percent R.S.D. recovery, accuracy, precision and bias.

RESULTS AND DISCUSSION

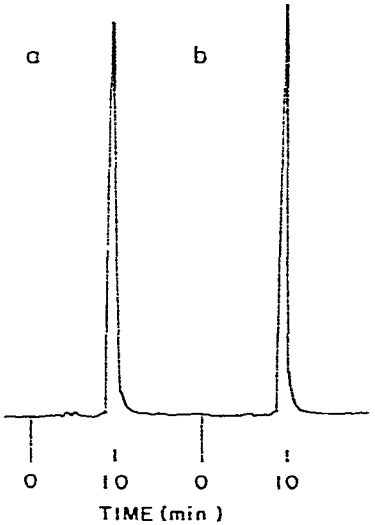
Linearity of recovery was obtained by the rapid extraction reversed-phase technique. Table II shows the results of duplicate sample analysis by peak height and peak area measurements. Peak heights generally proved to be most useful having percent recoveries in the 99.6–99.8% range and were consequently used in subsequent procedures. No interference from excipient peaks is seen in either the reversed-phase or the normal phase systems as seen in Figs. 2 and 3. The results of replicate analysis

TABLE II

LINEARITY OF PENTAZOCINE RECOVERY BY REVERSED-PHASE HPLC

Sample	*mg Pentazocine hydrochloride contained*	*mg Pentazocine hydrochloride found*	
		Peak height	*Peak area*
0%-1	0.0	0.0	0.0
0%-2	0.0	0.0	0.0
80%-1	24.2	24.4	24.3
80%-2	23.8	24.4	24.1
100%-1	28.4	28.4	29.1
100%-2	28.3	28.3	28.3
120%-1	32.2	31.3	30.2
120%-2	32.0	31.1	30.4
	Average % recovery	99.6	98.7
	% R.S.D. recovery	2.19	3.69
	Slope	0.985	0.972
	Intercept	+0.636	+0.921

of tablet mixtures by reversed-phase ion-pairing and by normal phase adsorption techniques show excellent correlation, Table III. While this correlation was obtained in terms of accuracies and precisions of recovery, overall simplicity and rapidity of the reversed-phase method makes it more practicable.



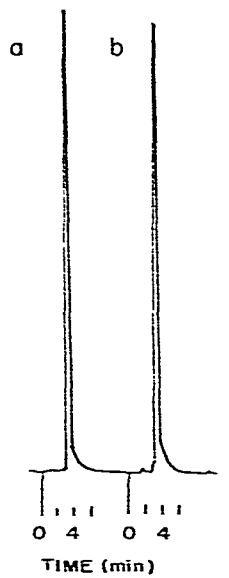


Fig. 2. Reversed-phase ion-pairing chromatograms of (a) pentazocine hydrochloride standard and (b) extracted tablet mixture of pentazocine hydrochloride. Mobile phase: sodium octanesulfonate (0.005 *M*)–methanol–H_3PO_4 (600:400:1). Stationary phase: Microbondapak C_{18}.

Fig. 3. Normal phase chromatograms of (a) pentazocine hydrochloride standard and (b) extracted tablet mixture of pentazocine hydrochloride. Mobile phase: chloroform–methanol–isopropylamine (960:40:2). Stationary phase: 5 μm silica.

TABLE III

REPLICATE ANALYSIS OF PENTAZOCINE, 25 mg

Reversed-phase ion-pairing			*Normal phase*		
mg Pentazocine hydrochloride		*Percent recovery*	*mg Pentazocine hydrochloride*		*Percent recovery*
Added	*Found*		*Added*	*Found*	
28.4	27.7	97.5	28.2	28.1	99.6
28.2	28.1	99.6	28.1	27.9	99.3
28.3	28.1	99.3	28.4	27.9	98.2
28.4	28.6	100.7	28.1	27.8	98.9
28.2	27.5	97.5	28.2	27.4	97.2
Average % recovery		98.9			98.6
% R.S.D. recovery		1.41			0.97

It was possible with the inexpensive UV detector utilized in the reversed-phase studies to detect a pentazocine peak at 1:1000 standard dilution. This had a peak height ratio of approximately 2 × baseline noise. This signal however was in the non-linear portion of the range. The linear range extended at least to the 1:100 standard dilution or 5.6 μg/ml of injected solution. Table IV shows these results along with information on the linear portion of the dilution plot. While the sensitivity of the present procedure could have been increased considerably by use of a lower wavelength of detection, such as 229 nm, or by a derivatization procedure, either is unwarranted in routine dosage form analysis of this compound.

Capacity factors for benzomorphan derivatives chromatographed are shown in Table V. These ranged from 1.4 to 5.8 having retention times from 4.8 to 13.5 min. These extreme values were found for the more polar norpentazocine and the less polar ketopentazocine respectively with pentazocine itself having a capacity factor of 4.0. Fig. 4 is a chromatogram of a mixture of pentazocine and pentazocine hydrate. This latter compound is one of the few known degradation products of pentazocine[19–21]. The separation achieved for various benzomorphans under the stated reversed-phase ion-pairing conditions is seen in Fig. 5.

TABLE IV

MINIMUM QUANTIFIABLE LIMIT

Correlation line using first three points of log peak height *vs.* log dilution factor +3: slope = 0.935; intercept = 2.29; $r = 0.999$; % R.S.D. of $y = 1.27$.

Pentazocine hydrochloride standard dilution	*Computer peak height observed (computer units)*
Initial (0.561 mg/ml)	130,805
1:10	13,183
1:100	1765
1:1000	542

TABLE V

CAPACITY FACTORS FOR BENZOMORPHANS

Solvent front retention time 1.8–2.0 min.

Benzomorphan	*k'*
nor-Pentazocine	1.4
Pentazocine hydrate	1.7
Cyclazocine hydrochloride	2.4
Ketocyclazocine	3.4
Pentazocine hydrochloride	4.0
Pentazocine acetate	4.0
Win 20722	4.4
Win 28389	4.5
Ketopentazocine	5.8

The variation in capacity factor for pentazocine hydrochloride and cyclazocine hydrochloride with number of carbons in the pairing ion is shown in Fig. 6. Here a break in the curves occurs between eight and ten carbons. Behavior of this nature has been recently reported for codeine, morphine and ethylmorphine[22]. Ion-pair formation in the mobile phase was postulated with increased retention being due to an increase in hydrophobicity of the ion-pairs. This results when the pairing ion of more than eight carbons length extends beyond the surface of the morphine derivative to which it was paired. This overlap of pairing ions can be similarly visualized in the case of benzomorphans by use of molecular models. The carbon chain of the pairing ion fits well on the α side of the ring system *trans* to the nitrogen bridge. Pairing ion carbons of eight or more extend beyond the benzene ring allowing for additional

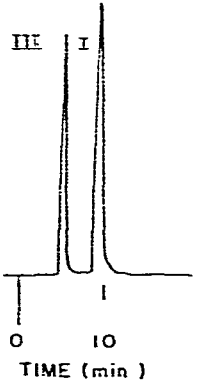


Fig. 4. Reversed-phase ion-pairing chromatogram of mixture of pentazocine hydrochloride (I) and its degradation product pentazocine hydrate (III). Chromatographic conditions given in Fig. 2.

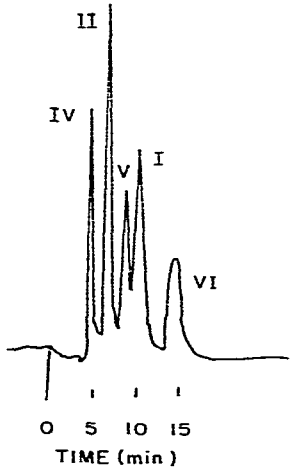


Fig. 5. Reversed-phase ion-pairing chromatogram of benzomorphan mixture including nor-pentazocine (IV), cyclazocine hydrochloride (II), ketocyclazocine (V), pentazocine hydrochloride (I) and ketopentazocine (VI). Chromatographic conditions given in Fig. 2.

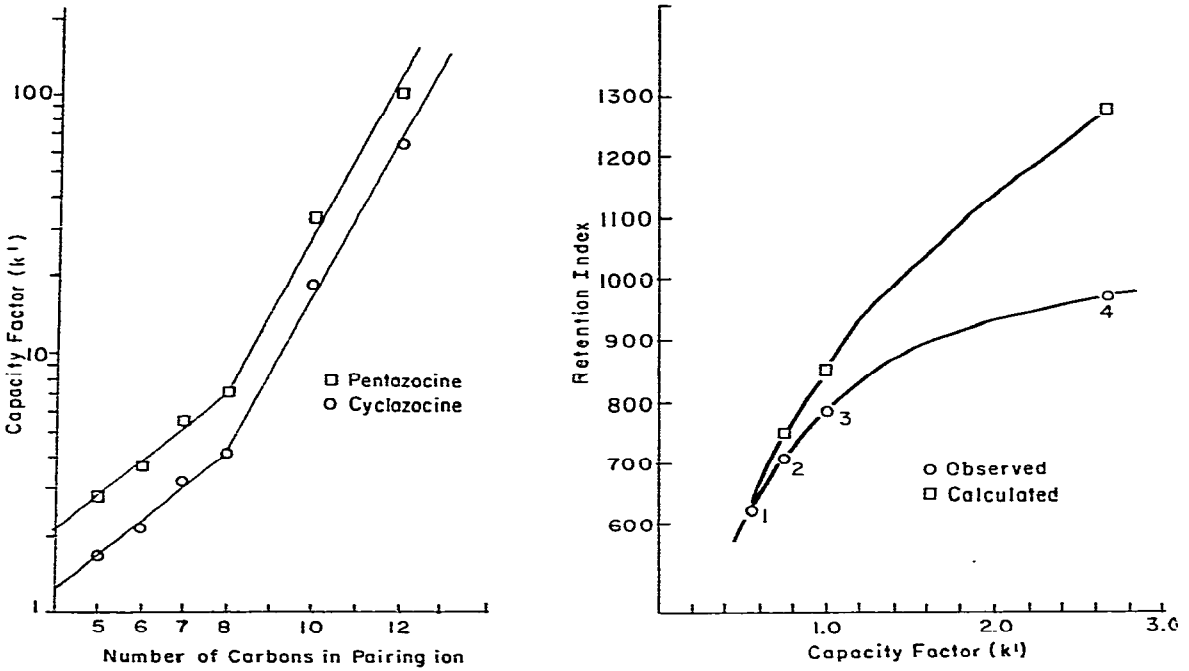


Fig. 6. Relationship between log capacity factor and number of carbons in pairing ion for pentazocine and cyclazocine. Mobile phase: sodium salt of pairing ion (0.005 *M*)–methanol–H_3PO_4 (600:600:1). Stationary phase: octadecylsilane.

Fig. 7. Relationship between calculated and observed retention indices (ref. 16) and measured capacity factors. 1 = Morphine, 2 = codeine and 3 = ethylmorphine (ref. 22), 4 = pentazocine, this study. Mobile phase: 0.005 *M* sodium octanesulfonate and 0.01 *M* ammonium nitrate in acetonitrile–water (375: 625). pH adjusted to 3.3 with acetic acid.

hydrophobic interaction with non-polar bonded phases. Ion-pairing of this nature has similarly been proposed by Loh *et al.*[23] between opiates and cerebroside sulfate, a model for the opiate receptor.

A decrease in capacity factors for pentazocine and cyclazocine to 2.7 and 1.8 respectively was obtained when chromatographed under the conditions of the earlier work[22] using the octanesulfonate pairing ion. This gives the same rank ordering as the retention index values calculated and measured in the non-pairing-ion reversed-phase study of Baker *et al.*[16]. In that study the calculated retention index, based on lipophilicity, of pentazocine was greater than the observed value. This indicates that partitioning behavior into the C_{18} bonded phase was less important under the conditions of their analysis. The non-linear nature of the relation between retention indices and observed capacity factors obtained by ion-pairing seen in Fig. 7 shows that different mechanisms are operative. The increased hydrophobicity of the formed ion-pair and a possible ion exchange component would contribute to this increased retention.

The results of the pairing ion concentration effect are seen in Fig. 8 where the customary "hyperbolic" relation between capacity factors for pentazocine and cycla-

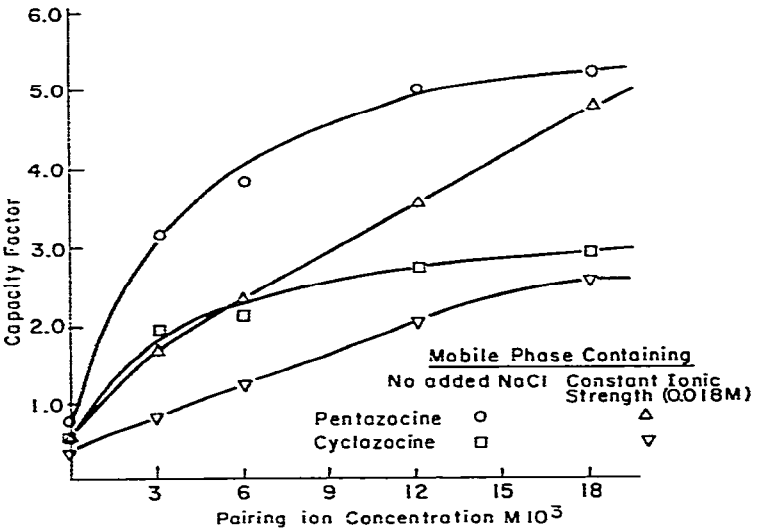


Fig. 8. Effect of sodium octanesulfonate pairing ion concentration on capacity factor for: pentazocine hydrochloride (○) and cyclazocine hydrochloride (□); pentazocine hydrochloride (△) and cyclazocine hydrochloride (▽) at constant ionic strength of 18 m*M* with added NaCl. Other chromatographic conditions as in Fig. 2.

zocine and the pairing ion concentration is apparent. While this relation can be explained by either ion-pair formation in the mobile phase or an ion exchange retention mechanism[24], it is umimportant to the actual chromatographic process according to other authors[25]. The decrease in capacity factor with increased ionic strength apparent in Fig. 8 has been previously observed for a series of benzoic acids[26]. This behavior can be explained by a decrease in the ion-pair formation constant at increased ionic strength, possibly due to a shielding effect of the added ions. This could support a retention mechanism such as the dynamic complex exchange of Melander and Horvath[24].

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