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SIMULTANEOUS HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF PROPOXYPHENE AND ACETAMINOPHEN IN PHARMACEUTICAL PREPARATIONS

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SUMMARY

A normal-phase high-performance liquid chromatographic method, using a 3.3 cm × 4.6 mm silica column (3 μm packing) and an ammonium hydroxide–methanol–dichloromethane mobile phase, has been developed to separate propoxyphene and acetaminophen in less than 5 min. Quantitative recovery of both compounds from various pharmaceutical preparations was achieved, following a single dilution scheme. The method is stable, reproducible, and selective.

INTRODUCTION

Propoxyphene (dextropropoxyphene, 1,2-diphenyl-2-propionoxy-3-methyl-4-dimethylaminobutane) is an analgesic commonly prescribed for the treatment of moderate pain. The need to quantify propoxyphene in biological fluids is brought about by its inherent addictability and the frequent occurrence of self-poisoning. The compound exists in two diastereoisomeric forms: α-*d,l* and β-*d,l*. The α-*d* isomer has analgesic properties; the α-*l* isomer has antitussive properties. The β isomers are unwanted, inactive contaminants of pharmacological preparations.

Barkan and Wainer[1] separated the α-*d* and β isomers of propoxyphene on a silica high-performance liquid chromatography (HPLC) column, using a water–isopropanol–hexane mobile phase. The content of β isomers in various commercial formulations was 0 to 0.45%. Angelo *et al.*[2] used a cyano HPLC column and an acetonitrile–methanol–10 m*M* sodium phosphate (pH 7) mobile phase to separate propoxyphene from its major metabolite, nordextropropoxyphene, in serum. The separation followed a relatively simple liquid–liquid extraction procedure. The problems associated with propoxyphene determination by gas chromatography have been discussed elsewhere[3].

Propoxyphene and acetaminophen (APAP, paracetamol, 4-acetamidophenol) are combined in various pain reliever formulations. A reliable simultaneous HPLC method for both compounds would be of interest to the manufacturers of these formulations. Such an analysis has been reported[4], but we could not reproduce the separation in our laboratory. Our goal was to separate propoxyphene and

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acetaminophen by isocratic elution in a short analyis time, while resolving the drugs from impurities and tablet excipients. The method should also yield good recoveries from dosage forms.

EXPERIMENTAL

Chemicals and reagents

All solvents were of HPLC grade and obtained from Accusolv Anachemia (Champlain, NY, U.S.A.). Deionized water was prepared in-house (Milli-Q system, Millipore, Milford, MA, U.S.A.). Reagent-grade concentrated ammonium hydroxide and HPLC-grade potassium dihydrogenphosphate were purchased from Fisher Chemical (Fair Lawn, NJ, U.S.A.). Triethylamine was obtained from Kodak (Rochester, NY, U.S.A.).

The acetaminophen and/or propoxyphene content was determined for the following dosage forms obtained from a local pharmacy: Darvon-N® (100 mg propoxyphene napsylate, Eli Lilly & Co.), Darvocet-N® 100 (100 mg propoxyphene napsylate, 650 mg acetaminophen, Lilly), and generic propoxyphene N 100 APAP (100 mg propoxyphene napsylate, 650 mg acetaminophen).

Propoxyphene hydrochloride standard (United States Pharmacopeia, Rockville, MD, U.S.A.) was dried at 105°C for 3 h prior to use. Acetaminophen standard and the acetaminophen-related compounds, *p*-nitrophenol, *p*-hydroxyacetophenone, and 4-aminophenol, were obtained from Sigma (St. Louis, MO, U.S.A.).

Chromatographic system

The chromatographic system employed an HP-1090 liquid chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) equipped with an autosampler, column oven, HP-85B system controller, HP-3392A integrator/recorder, and a HP 1040A diode array detector set at 220 or 244 nm UV with a response time of 160 ms. All Supelcosil® HPLC columns (3.3 cm × 4.6 mm I.D., 3-μm packings) were obtained from Supelco, Bellefonte, PA, U.S.A. Analytical columns were protected by guard columns (2 cm × 4.6 mm I.D., 5-μm packings) containing the same phase. In some experiments, small-bore (3.3 cm × 2.1 mm I.D.) columns packed with 3-μm silica were used to conserve mobile phase.

Chromatography

The standard stock solution for construction of the calibration curve was prepared by dissolving 10 mg propoxyphene and 100 mg acetaminophen in 2 ml of methanol in a 50-ml volumetric flask and diluting to the mark with dichloromethane. This stock solution was quantitatively diluted in mobile phase to yield six solutions ranging from 20 to 80 μg/ml propoxyphene and 200 to 800 μg/ml acetaminophen. These solutions bracketed the concentrations of the drugs in the final dilution from the dosage forms. Calibration curves for each compound were generated by making triplicate 10-μl injections of each of the standard solutions. Average peak area or height was plotted versus concentration.

One Darvocet-N 100 tablet, generic propoxyphene N 100 APAP tablet, or Darvon capsule was added to each of six 100-ml volumetric flasks. A 10-ml volume of methanol and 80 ml of dichloromethane were added to each flask. The solutions were

sonicated for 5 min and, after cooling to room temperature, diluted to the mark with dichloromethane. These solutions were quantitatively diluted 2/25 in mobile phase to yield solutions of 520 μg/ml acetaminophen and/or 52 μg/ml propoxyphene.

Average peak area or height was obtained from triplicate 10-μl injections of final tablet or capsule dilutions. Recovery of the active ingredient (percentage of label claim) was obtained by interpolating the calibration curves.

In addition, three acetaminophen decomposition products or synthesis precursors (*p*-nitrophenol, *p*-hydroxyacetophenone and 4-aminophenol) were investigated for possible interference with the drug analysis. Interference from dosage form excipients was also investigated.

The mobile phase for analysis on silica HPLC columns consisted of ammonium hydroxide in methanol (3.33%, v/v)–dichloromethane (1.5:98.5). It is important to store all reagents and solvents at the same temperature, prior to mixing, to prevent volume/temperature effects.

Retention behavior of propoxyphene and acetaminophen was studied on silica, cyano and diol polar phases under various mobile phase conditions. After establishing a suitable HPLC method, changes in retention of propoxyphene and acetaminophen over time, with increasing mobile phase volume, were investigated to evaluate the stability of the system. In some experiments, the mobile phase was recycled by inserting the outlet from the detector into the solvent reservoir, after approximately ten column-volumes had passed through the system. Small-bore columns were used in this portion of the experiment to reduce mobile phase consumption. Reproducibility of the analysis was investigated by using a total of five columns representing three lots of silica.

RESULTS AND DISCUSSION

Assay procedure

Propoxyphene and acetaminophen were separated isocratically in less than 5 min on a 3.3-cm column containing 3-μm silica using ammonium hydroxide in methanol (3.33%, v/v) dichloromethane (1.5:98.5) as the mobile phase. Typical chromatograms showing the two compounds extracted from commercial tablets are shown in Fig. 1. The sample solvent must contain at least 1.5% methanol to maintain

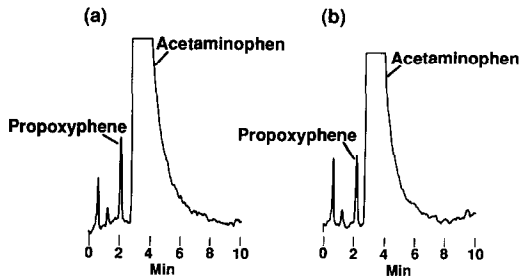

Fig. 1. Drug analysis on silica. Column: 3.3 cm × 4.6 mm Supelcosil LC-Si (3-μm packing) with guard column, mobile phase ammonium hydroxide in methanol (3.33%, v/v)–dichloromethane (1.5:98.5), 2 ml/min, 30°C, detection 244 nm 0.004 a.u.f.s., 10-μl injection volume (52 μg/ml propoxyphene, 520 μg/ml acetaminophen). (a) Darvocet-N 100 extract; (b) generic propoxyphene N 100 APAP extract.

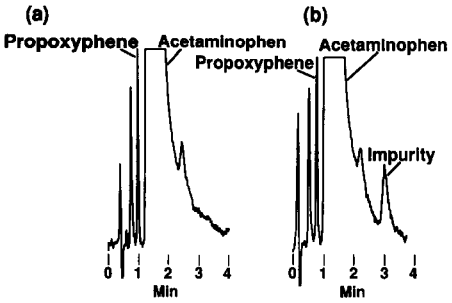

Fig. 2. Drug analysis on silica. Conditions: as in Fig. 1 except mobile phase ammonium hydroxide in methanol (1.67%, v/v)–dichloromethane (3:97). (a) Darvocet-N 100 extract; (b) generic propoxyphene N 100 APAP extract.

solubility of acetaminophen, but no more than 2.5% methanol to prevent interference of the solvent front with the propoxyphene peak. By increasing the amount of methanol in the mobile phase, an unidentified peak was revealed in the generic formulation that was not detected in the Darvocet-N 100 formulation (Fig. 2).

Extraction of propoxyphene and acetaminophen from various commercial formulations was complete and without interference from excipients. Recovery data for both compounds are given in Table I. Propoxyphene and acetaminophen response was linear for both peak height and area *versus* concentration over the investigated concentration ranges. Despite the wide disparity between propoxyphene and acetaminophen concentrations in the dosage forms, the linear acetaminophen response at high levels ensured the drugs could be simultaneously assayed through a single dilution scheme. Calibration curve data for acetaminophen and propoxyphene are given in Table II.

The normal-phase analysis is sensitive. At 244 nm UV the limits of detection were 25 and 1 ng, on-column quantities of propoxyphene and acetaminophen, respectively, with a signal-to-noise ratio of 2:1.

None of the acetaminophen-related compounds interfered with propoxyphene or acetaminophen quantification. *p*-Nitrophenol elutes in 0.71 min, *p*-hydroxyacetophenone in 0.72 min, and 4-aminophenol in 1.03 min. Two unidentified impurities in the acetaminophen preparations elute at 1.05 and 1.85 min. Impurities in acetaminophen preparations have been studied by others[5,6].

TABLE I

RECOVERY OF PROPOXYPHENE AND ACETAMINOPHEN FROM DOSAGE FORMS

Preparation	*Recovery (%)*	
	Propoxyphene	*Acetaminophen*
Darvocet-N 100	96.7 ± 4.8%	102.4 ± 1.7%
Propoxyphene N 100 APAP	92.2 ± 2.2%	102.0 ± 0.9%
Darvon	105.4 ± 4.2%	–

TABLE II

STANDARD PEAK AREA OR PEAK HEIGHT *VS.* CONCENTRATION CURVES FOR PROPOXYPHENE AND ACETAMINOPHEN UNDER VARIOUS CONDITIONS

$y = mx + b$; r = coefficient of correlation.

Compound	*Concentration range (μg/ml)*	*r*	*m*	*b*
Propoxyphene	20–160	0.9988	750 area counts/(μg/ml)	−3137 area counts
	20–80	0.9988	0.03889 cm/(μg/ml)	0.283 cm
Acetaminophen	200–800	0.9992	58083 area counts/(μg/ml)	$-1.29 \cdot 10^6$ area counts

TABLE III

RETENTION OF ACETAMINOPHEN AND PROPOXYPHENE ON NORMAL-PHASE COLUMNS IN VARIOUS MOBILE PHASES

k' = Capacity factor.

Column	*Mobile phase*	$k'_{Propoxyphene}$	$k'_{Acetaminophen}$
Diol	Isopropanol–hexane (60:40)	>20	0.14
	Isopropanol–hexane (72:28)	>20	0.10
	Ethylacetate–hexane (35:65)	>20	>20
	Dichloromethane	>20	3.5
	Dichloromethane–tetrahydrofuran (99:1)	1.1	–
	Water–methanol–dichloromethane (0.1:0.5:99.4)	0.25	1.5
	Water–methanol–dichloromethane (0.1:0.5:99.4) + 0.05% ammonium hydroxide	0	1.4
Cyano	Isopropanol–hexane (60:40)	0	0.1
	Isopropanol–hexane (50:50)	2.7	–
	Isopropanol–hexane (50:50) + 2% tetrahydrofuran	3.4	0.18
	Isopropanol–hexane (20:80) + 0.02% ammonium hydroxide	>20	1.6
	Isopropanol–hexane (20:80) + 0.01 ammonium hydroxide	>20	0.25
	Dichloromethane	>20	>20
	Water–methanol–dichloromethane (0.1:0.5:99.4)	>20	>20
	Methanol–dichloromethane (1.5:98.5) + 0.05% ammonium hydroxide	0	0
	Acetonitrile–dichloromethane (2.5:97.5) + 0.05% ammonium hydroxide	3.7	12.0
Silica	Isopropanol–hexane (25:75)	0	0.1
	Isopropanol–hexane (10:90)	0	5.9
	Dichloromethane	>20	–
	Acetonitrile–dichloromethane (2:98) + 0.05% ammonium hydroxide	>20	>20
	Isopropanol–dichloromethane (2:98) + 0.05% ammonium hydroxide	>20	>20
	Methanol–dichloromethane (2:98) + 0.02% triethylamine	>20	5.1
	Methanol–dichloromethane (2:98) + 0.2% triethylamine	>20	10.8
	Methanol–dichloromethane (2:98) + 0.05% ammonium hydroxide	2.6	7.1
	Methanol–dichloromethane (1.5:98.5) + 0.05% ammonium hydroxide	5.2	10.0

Investigation into retention mechanism

Retention behavior of propoxyphene and acetaminophen on silica, cyano and diol polar phase columns under various mobile phase conditions is summarized in Table III. The mobile phase chosen for separating propoxyphene and acetaminophen on a silica column was too strong for use with a cyano column. Substituting acetonitrile for the methanol, however, resulted in satisfactory separation on the cyano column. Peak shapes obtained on the cyano column, however, were not as good as those obtained on the silica column, and equilibration time was too long. Further experimentation on the cyano column was not pursued. Mobile phase conditions for the simultaneous analysis of both drugs on a diol column could not be found.

The effect of changes in the ammonium hydroxide concentration on retention of the two drugs on silica and cyano columns is shown in Fig. 3. Ammonium hydroxide was needed in the mobile phase to elute propoxyphene, a tertiary amine, from the silica

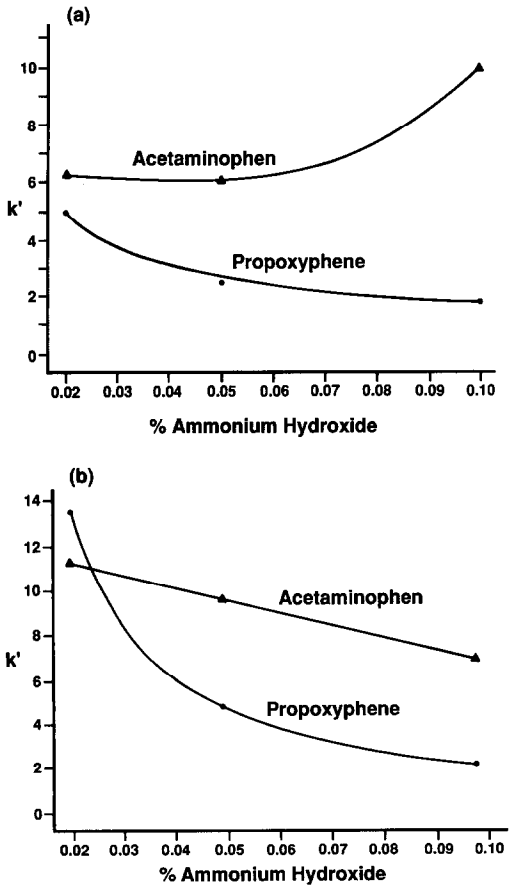

Fig. 3. Retention of propoxyphene and acetaminophen as a function of ammonium hydroxide concentration in mobile phase. (a) Silica column, mobile phase ammonium hydroxide in methanol (1–5%)–dichloromethane (2:100); (b) cyano column, mobile phase ammonium hydroxide in acetonitrile (0.8–4%)–dichloromethane (2.5:97.5).

column. Without ammonium hydroxide, 7% methanol in dichloromethane was required for elution of propoxyphene from a silica column. Acetaminophen, however, was not retained under these conditions. By adding a small amount of ammonium hydroxide to the mobile phase, the percentage of methanol could be decreased and both drugs analyzed simultaneously. Concentrations of 1.5% methanol and 0.05% ammonium hydroxide in dichloromethane produces good k' values and sharp, symmetrical peaks for both drugs.

On the silica column, ammonium hydroxide forms strong hydrogen bonds with surface silanols. Thus, increasing its concentration decreases the number of silanols available for binding of the weakly basic propoxyphene. This decreases the retention of propoxyphene while also improving peak shape.

Acetaminophen, a weak acid, and ammonium hydroxide undergo ion-pair interaction forming a complex that can undergo weak hydrogen bonding with the surface silanols. For a complete treatment of silanol interaction see Unger[7].

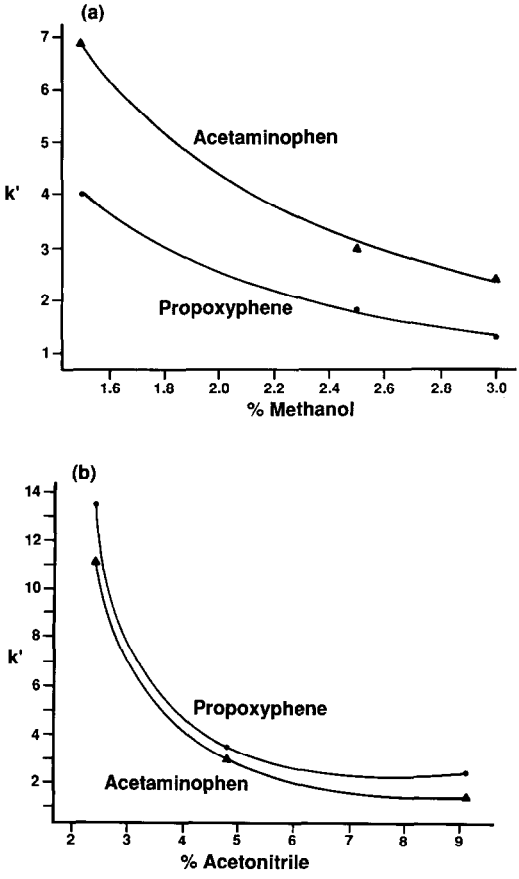

Fig. 4. Retention of propoxyphene and acetaminophen as a function of polar organic modifier concentration in mobile phase. (a) Silica column, mobile phase 1.5–3% methanol in dichloromethane, 0.05% ammonium hydroxide; (b) cyano column, mobile phase 2.4–9.1% acetonitrile in dichloromethane, 0.02% ammonium hydroxide.

We also established that propoxyphene would not elute from a silica column if triethylamine and water (to promote ionization of the triethylamine) replaced the ammonium hydroxide in the mobile phase. The use of ionic eluents as mobile phase additives, for analysis of basic compounds on unmodified silica, has been studied elsewhere[3,8,9].

While hydrogen bonding is the dominant mechanism in retention by unmodified silica, dipole interactions become important with cyano-modified silica in a normal-phase mode of operation (non-aqueous environment). With the cyano-bonded phase, however, interaction with residual silanols can also occur. These silanols have high polarity relative to the nitrile moieties of the bonded phase. It is therefore likely that the change in retention with increasing ammonium hydroxide concentration seen for both drugs is due mostly to the effect of ammonium hydroxide on the activity of the residual silanols.

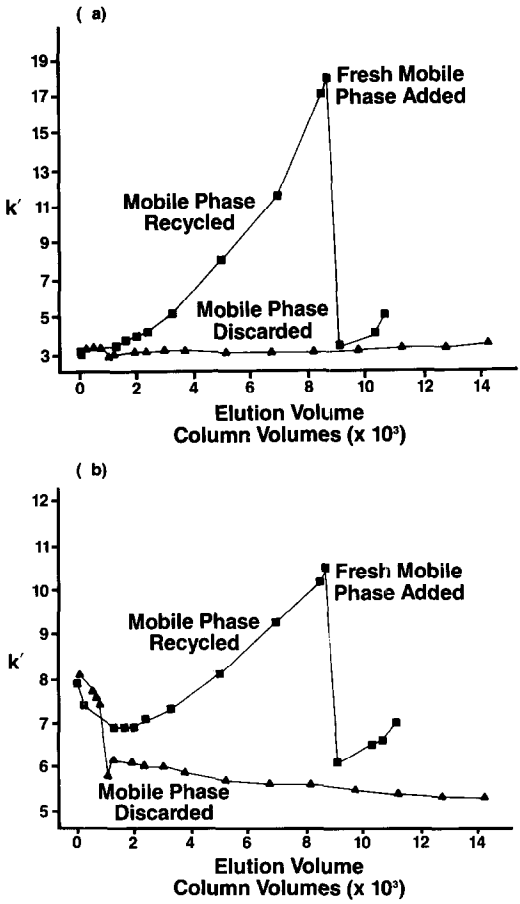

Fig. 5. Effect of mobile phase volume passed through column on retention of (a) propoxyphene and (b) acetaminophen by silica column. Mobile phase: ammonium hydroxide in methanol (3.33%)–dichloromethane (1.5:98.5).

The effect of varying the polar organic modifier (methanol or acetonitrile) concentration is shown in Fig. 4. As expected, both propoxyphene and acetaminophen retention decreased with increasing competing organic modifier concentration. This behavior is consistent with a normal-phase mechanism on both the silica and cyano columns (see ref. 10). Methanol was a stronger polar modifier than isopropanol and acetonitrile for elution of both acetaminophen and propoxyphene from the silica column.

Stability of retention

Changes in retention of acetaminophen and propoxyphene on a 3-μm silica column as a function of number of column volumes of mobile phase passed through the column can be seen in Fig. 5. Mobile phase leaving the column was either discarded (conventional) or recycled. The baseline usually stabilized within ten column volumes (5–10 ml) for a new column.

With mobile phase pumped in the conventional mode, acetaminophen shows a rapid decrease in retention over the first 200 column volumes (100 ml), and a shallow but steady decrease thereafter. Retention of propoxyphene changes only slightly. The drift in retention of acetaminophen does not adversely affect its resolution from propoxyphene for at least 10 000 column volumes (Fig. 6). This represents 48 h of continuous use, or 1000 analyses.

Recycled and conventional mobile phase operation yields similar retention for acetaminophen and propoxyphene over the first 200 column volumes. After this volume of recycled mobile phase, retention for both compounds increases steadily, leading to coelution and peak reversal at about 4000 column volumes. The separation could be returned to normal by pumping fresh mobile phase through the column (Figs. 5 and 6).

An apparent drawback to mobile phase recycling is the gradual loss of transmission of UV light through the detector cell. Flushing the cell with methanol will restore the output, but the baseline may take a great deal of time to restabilize. Flushing the column with methanol does not hasten the stabilization of the baseline.

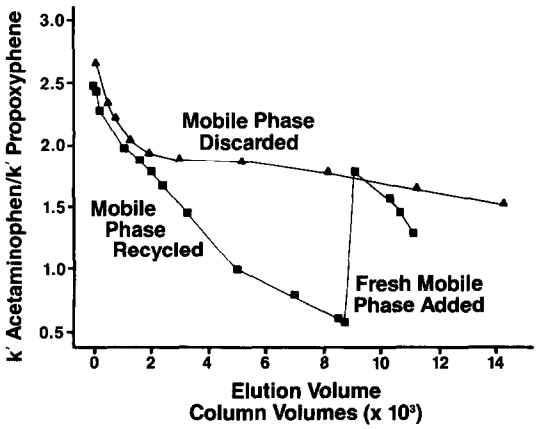

Fig. 6. Effect of mobile phase volume passed through column on separation factor on silica column. Mobile phase: ammonium hydroxide in methanol (3.33%)–dichloromethane (1.5:98.5).

TABLE IV

REPRODUCIBILITY OF NORMAL PHASE ANALYSIS FOR PROPOXYPHENE AND ACETAMINOPHEN

Column: 3.3 cm × 4.6 mm silica, 3-μm packing; mobile phase: ammonium hydroxide in methanol (3.33%, v/v)–dichloromethane (1.5:98.5); flow-rate: 2.0 ml/min.

Silica lot and column number	*k'*					
	Propoxyphene			*Acetaminophen*		
	Initial	*After 0.2 l mobile phase*	*After 1 l mobile phase*	*Initial*	*After 0.2 l mobile phase*	*After 1 l mobile phase*
Lot A, column 1 (recycled mobile phase)	4.0	4.0	4.0	10.2	9.0	7.8
Lot B, column 2	3.7	3.7	3.6	9.8	8.8	7.5
Lot B, column 3	3.8	3.8	3.6	10.4	9.6	6.9
Lot B, column 4 (recycled mobile phase)	3.8	3.7	3.7	9.8	8.6	7.3
Lot C, column 5	5.7	4.0	3.9	11.6	8.4	7.4

Decreased detector output was also seen when stagnant mobile phase was left in the detector cell (in both normal and recycled modes).

Reproducibility

The normal-phase separation we developed was reproduced on five columns, representing three silica lots, without the need for mobile phase adjustment. Table IV shows the *k'* values obtained initially, after 200 ml, and after 1 l of mobile phase had passed through the column. Capacity factors were consistent from column-to-column and lot-to-lot. Column 5 from Lot C showed initially higher *k'* values for both compounds, but after a short equilibration time (10 column volumes), became consistent with the other columns.

CONCLUSION

A method to simultaneously quantify propoxyphene and acetaminophen from dosage forms has been developed. The system employs a 3.3 cm × 4.6 mm column containing 3-μm silica, and an ammonium hydroxide in methanol (3.3%, v/v)–dichloromethane mobile phase (1.5:98.5). The method has been shown to be stable, sensitive and reproducible.

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