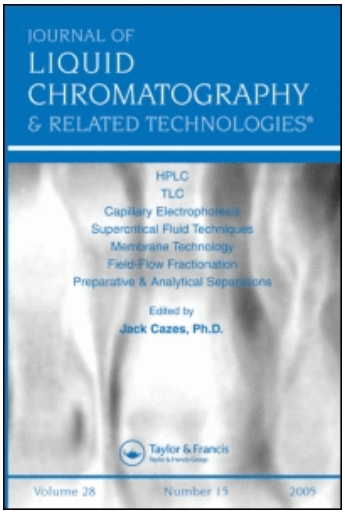

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HPLC METHODS FOR ASPIRIN-CAFFEINE-BUTALBITAL AND ACETAMINOPHEN-CAFFEINE-BUTALBITAL MIXTURES IN TABLET DOSAGE FORMS USING NON-POROUS OCTADECYLSILANE COLUMNS

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ABSTRACT

High performance liquid chromatography procedures using non-porous ODS columns were developed for the assay of aspirin-caffeine-butalbital (mixture 1) and acetaminophen-caffeine-butalbital (mixture 2). The separation and quantitation of Mixture 1 were achieved on a 3.0 μm non-porous silica ODS column at ambient temperature using a mobile phase of 98:2 v/v 50 mM phosphate buffer pH 3.0-acetonitrile at a flow rate of 1.5 mL/min with detection at 220 nm. The method showed linearity for aspirin-caffeine-butalbital in the 325-6500, 40-800, and 50-1000 ng/mL ranges, respectively. Intra- and inter-day RSD values were 0.19-1.72% and 1.30-1.49% for aspirin, 0.08-1.17% and 0.06-1.09% for caffeine, and 0.09-1.55% and 0.07-2.10% for butalbital, respectively. Accuracy of intra and inter-day were in the 0.70-1.27% and 0.20-1.13% for aspirin, 0.05-0.06% and 0.004-0.09% for caffeine, and 0.02-0.09 and 0.02-0.05% for butalbital, respectively.

The limits of detection for aspirin, caffeine, and butalbital were 5, 5, and 10 ng/mL, respectively, based on a signal noise ratio of

3 and a 50 μL injection. The separation and quantitation of mixture 2 were achieved on a 3.0 μm non-porous silica ODS column at ambient temperature using a mobile phase of 97:3 v/v 50 mM phosphate buffer pH 2.5-methanol at a flow rate of 2.0 mL/min with detection at 220 nm. The method showed linearity for acetaminophen, caffeine, and butalbital in the 650-6500, 100-800, and 125-1000 ng/mL ranges, respectively. Intra- and inter-day RSD values were 0.06-2.64% and 2.18-4.19% for acetaminophen, 2.24-4.76% and 4.09-4.12% for caffeine, and 1.65-3.94% and 3.33-3.40% for butalbital, respectively. Accuracy of intra and inter-day were in the 1.13-2.86% and 0.08-0.10% for acetaminophen, 1.12-4.62% and 0.04-2.75% for caffeine, and 0.34-3.48% and 0.02-2.30% for butalbital, respectively. The limits of detection for acetaminophen, caffeine, and butalbital were 20, 20, and 35 ng/mL, respectively, based on a signal noise ratio of 3 and a 10 μL injection.

INTRODUCTION

Non-porous silica supports were first prepared by Unger et al.[1] and Stober et al.[2] Larry et al.[3] investigated the application of a nonporous silica (NPS) support to high performance liquid chromatography and predicted application even to the separation of macromolecules. Recent reports[4-7] have shown applications of NPS supports to chromatographic separations of small molecules, such as pharmaceuticals. Investigators have demonstrated that a reduced organic modifier content in the mobile phase can result in a significant decrease in chromatographic run time while preserving peak resolution. These findings are important in that these non-porous columns meet not only the environmentally friendly resolution of the 1995 USP Convention, but also can play a role in increasing workloads of many analytical laboratories.

Aspirin-caffeine-butalbital (Mixture 1) and acetaminophen caffeine-butalbital (Mixture 2) are available in tablet dosage forms used for the relief of moderate to moderately severe pain[8]. These mixtures have been analyzed using near-IR[9] and HPLC Methods.[10-12] Presently, in USP 23, these types of tablet dosage forms are analyzed by reverse phase HPLC using porous ODS columns at elevated temperatures.[13] Run times of 10-20 min using high organic modifier ratios (>40%) in the mobile phase are typical.

This paper describes new isocratic HPLC methods to simultaneously determine each medication in mixtures 1 and 2 using nonporous ODS columns with run times within 5 min at ambient temperature (23 ± 1°C). The sensitivities of the HPLC methods extend to the ng/mL range for each component.

EXPERIMENTAL

Reagents and Chemicals

The chemical structures of the analytes studied are shown in Figure 1. Butalbital was obtained from Sigma (Lot No. 78F0535, St. Louis, MO, USA). Acetaminophen, aspirin, caffeine, and sodium salicylate powders were purchased from Sigma Chemical Company. All materials were used as received. The solvents used were HPLC grade and water was purified by a cartridge system (Picotech Water System, Research Triangle Park, NC, USA). Acetonitrile, absolute methanol, concentrated phosphoric acid, and potassium dihydrogenphosphate were obtained from J. T. Baker, Inc. (Phillipsburg, NJ, USA).

All chromatographic solutions were filtered through a 0.22 μm Nylon filter (Sigma, St. Louis, MO, USA, Lot No. 77H1734). Tablets of Mixture 1 (Fiorinal™, Lot No. 779W6330) and Mixture 2 (Fioriset™, Lot No. 134 W 6142) were manufactured by Novartis (East Hanover, NJ) and were purchased at a local pharmacy.

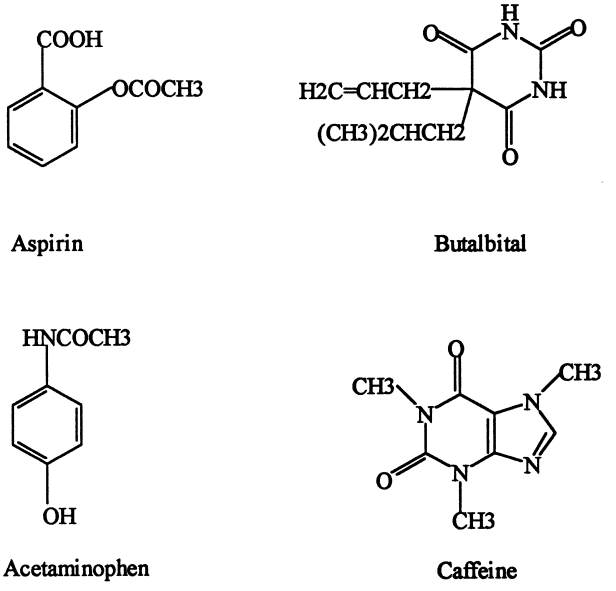

Figure 1. Chemical structures of aspirin, acetaminophen, butalbital and caffeine.

Instrumentation and Chromatographic Conditions

An HPLC system consisting of a Model 510 pump, a Model 996 Photodiode Array Detector set at 220 nm, a Digital Computer with Millennium software (version v 2.1 5.01, Waters Inc., Milford, MA, USA), and a Rheodyne Model 7125 injection valve (Rheodyne, Cotati, CA, USA) equipped with either a 20 μL (Mixture 1) or 10 μL (Mixture 2) sample loop was used. Nonporous silica (NPS) octadecylsilane columns (ODSI, II and IIE, 33 mm x 4.6 mm id, 3 μm and ODSI, ODSII, and ODSIIE 100 mm x 4.6 mm I.D., 3.0 μm) were supplied by MICRA Scientific, Inc. (Northbrook, IL, USA). The mobile phase for Mixture 1 was acetonitrile: aqueous 50 mM potassium dihydrogen phosphate buffer pH 3.0 (2:98, v/v). The mobile phase for Mixture 2 was methanol: aqueous 50 mM potassium dihydrogen phosphate buffer pH 2.5 (3:97, v/v). The chromatographic methods were performed at ambient temperature (23 ± 1°C) at a flow rate of 1.5 mL/min for Mixture 1 and 2 mL/min for Mixture 2.

Preparation of Stock Solutions

Individual stock solutions (1 mg/mL) containing aspirin, caffeine, butalbital, and acetaminophen were prepared by weighing appropriate amounts of each compound into volumetric flasks followed by dissolution with absolute methanol. The solutions were stable for at least two weeks at 4°C. Appropriate dilutions of the stock solutions with purified water gave concentrations of 325 μg/mL for aspirin and acetaminophen, 40 μg/mL for caffeine, and 50 μg/mL for butalbital. The appropriate mobile phases used for dilution of the stock solutions were also used for method development, preparation of calibration curves, and calculations of precision and accuracy.

Preparation of Calibration Curves

Various aliquots of the stock solutions were diluted with the appropriate mobile phase to provide calibration ranges for aspirin (325-6500 ng/mL), caffeine (40-800 ng/mL), and butalbital (50-1000 ng/mL) in Mixture 1, and acetaminophen (650-6500 ng/mL), caffeine (80-800 ng/mL), and butalbital (100-1000 ng/mL) in Mixture 2. Quantitation was based on linear regression analysis of peak height (Mixture 1) or peak area (Mixture 2) vs analyte concentration in ng/mL.

Analysis of Commercial Tablet Dosage Forms

Sample preparation of commercial tablets containing aspirin, caffeine, and butalbital (Mixture 1) and acetaminophen, caffeine, and butalbital (Mixture 2) were performed using the procedures described in the current USP 23 mono-

graph. For Mixture 1, ten tablets were ground in a mortar with a pestle and the powder equivalent to one average tablet weight (530 mg) was dissolved in 200 mL of phosphate buffer (0.01M, pH 2.5) in an ultrasonic bath for 30 min with stirring. The appropriate concentrations of analytes in the extract were aspirin (1.625 mg/mL), caffeine (200 μg/mL) and butalbital (250 μg/mL). An aliquot of 20 μL of the tablet extract was diluted to 10 mL with mobile phase prior to injecting into the HPLC system.

For mixture 2, ten tablets was ground in a mortar with a pestle and the powder equivalent to one average tablet weight (520 mg) was dissolved in 200 mL of absolute methanol in an ultrasonic bath for 15 min and allowed to cool. Twenty mL of the supernatant was transferred to a 50 mL volumetric flask, diluted with mobile phase to volume, and mixed. The final concentration of the analytes were approximately acetaminophen (650 μg/mL), caffeine (80 μg/mL), and butalbital (100 μg/mL). An aliquot (10 μL) of the tablet extract was diluted to 1 mL with mobile phase before injecting into the HPLC system.

RESULTS AND DISCUSSION

There are several advantages of using nonporous columns for HPLC separations. First, they show a higher sensitivity compared to porous columns. Second, at the flow rates used, waste solvent production is reduced by at least 50% and organic waste is reduced by 90%. However, nonporous columns are more susceptible to events that disturb the column equilibrium than conventional porous columns. Thus the solvent used to dissolve an analytical sample should have identical or closely similar composition to the mobile phase to avoid poor peak shapes. Faster analysis times and the ability to significantly decrease amounts of organic modifier could potentially allow for the replacement of porous by non-porous columns in many pharmaceutical applications.

In our initial studies, three different types of nonporous ODS columns were investigated. They were classified as ODSI (polymeric), ODS II (monomeric), and ODS IIE (monomeric and endcapped) available in both 33 and 100 mm lengths. The 33 mm ODS I, II, and IIE columns would not tolerate high flow rates of 1.5-2 mL/min or provide enough separation between analyte(s) and the solvent front. Although the interaction between analytes and the silica surface was less with a nonporous column, small molecules with different polarities showed wide differences in retention times. For example, a 97% aqueous mobile phase containing acetonitrile separated acetaminophen from the solvent front with good baseline resolution and gave a retention time for butalbital of less than 5 min. A 100% aqueous mobile phase gave no change in the retention time of acetaminophen but butalbital was not eluted within 30 min. Exchanging methanol for acetonitrile in the mobile phase resulted in the acetaminophen peak coeluting at the solvent front.

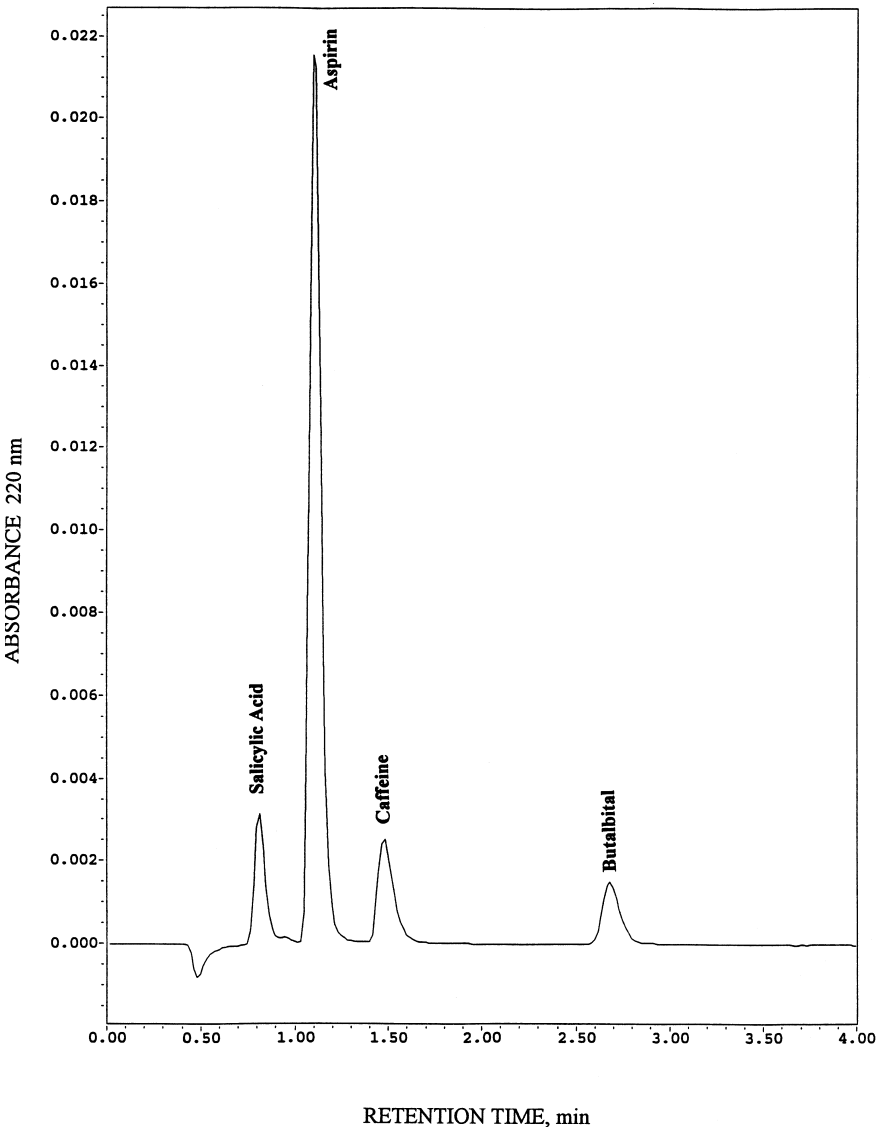

Figure 2. Typical HPLC separation of aspirin, caffeine and butalbital on 100 mm x 4.6 mm id NPS ODSII column. See Experimental Section for chromatographic parameters.

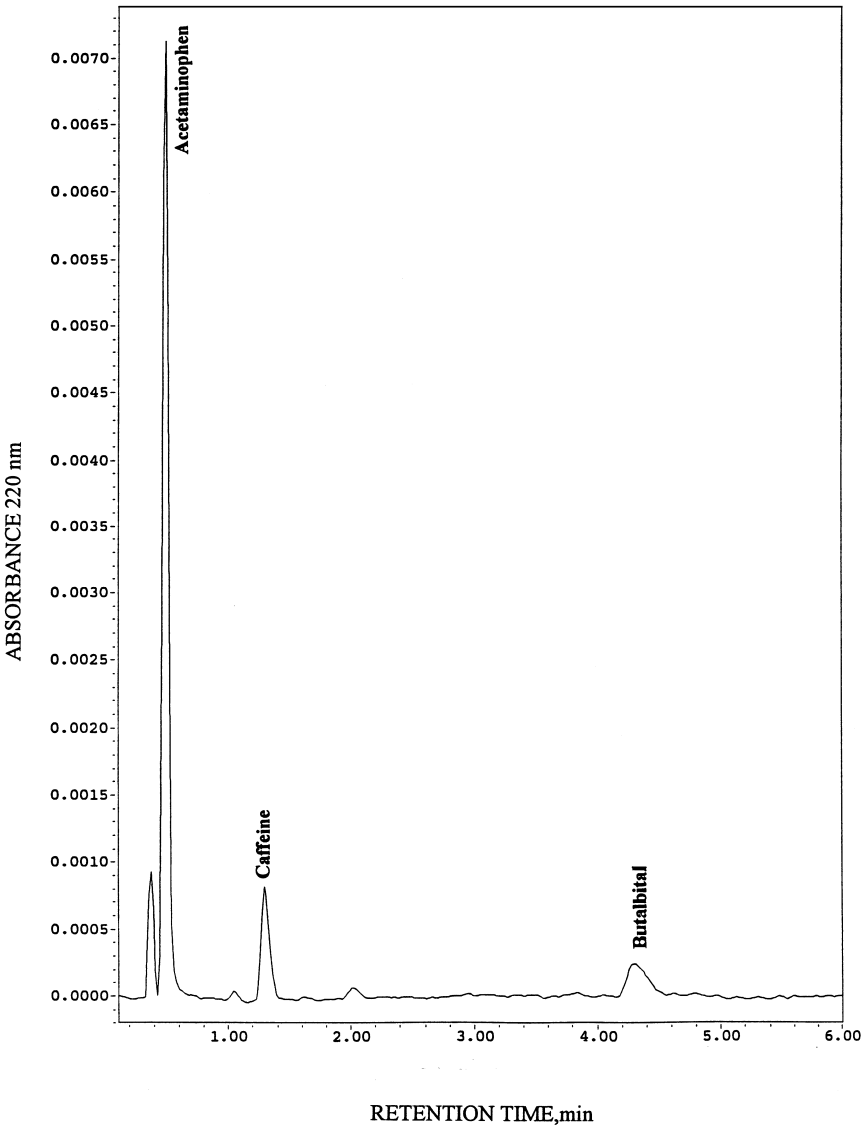

Figure 3. Typical HPLC separation of acetaminophen, caffeine and butalbital on 100 mm x 4.6 mm id NPS ODSIIE column. See Experimental Section for chromatographic parameters.

The monomeric ODSII (100 mm) NPS column provided suitable retention times for butalbital, due to less interaction between the drug and the carbon load compared to the ODSIIE column. It was shown that the ODSII column gave the best separation for Mixture 1. A typical HPLC chromatogram of the separation of aspirin, caffeine and butalbital on this column is shown in Figure 2. When analyzing Mixture 1, neither the polymeric nor monomeric NPS columns aided in better symmetry for the caffeine peak. Acetonitrile is a stronger organic modifier than methanol and provided symmetrical peak shapes with less tailing and a much lower pump back pressure. The ODSIIE column was selected for the separation of Mixture 2 since there was minimal interaction between the analytes and the column. A typical chromatogram of the separation of acetaminophen, caffeine, butalbital, on the ODSIIE column is shown in Figure 3.

The analytical figure of merit data for Mixtures 1 and 2 are shown in Table 1. For mixture 1, intra- and inter-day RSD values were 0.19-1.72% and 1.30-

Table 1

Analytical Figures of Merit for Aspirin, Caffeine, and Butalbital (Mixture 1) and for Acetaminophen, Caffeine, and Butalbital (Mixture 2)

Analyte	r^{2}[a]	System Suitability[b]	LOD[c] ng/mL	k	Theoretical Plates[d]	Tailing Factors[e]
Mixture 1						
Aspirin	0.9995	0.074	5	1.28	1337	1.05
Caffeine	0.9996	0.337	5	2.06	1502	1.4
Butalbital	0.9995	0.550	10	4.51	2500	1.0
Mixture 2						
Acetaminophen	0.9988	3.94	20	0.448	130	1.1
Caffeine	0.9991	1.97	20	2.457	782	1.1
Butalbital	0.9989	0.972	35	10.46	2393	1.05

[a] Range examined from 325-6500 ng/mL aspirin, 50-1000 ng/mL butalbital and 40-800 ng/mL caffeine in Mixture 1 and 650-6500 ng/mL acetaminophen, 100-1000 ng/mL, butalbital and 80-800 ng/mL caffeine in Mixture 2; (n=5). [b] RSD% of 5 replicate injections of Mixture 1 solutions containing 3250 ng/mL aspirin, 500 ng/mL butalbital, 400 ng/mL caffeine at 220 nm and Mixture 2 solutions containing 3250 ng/mL acetaminophen, 500 ng/mL butalbital, 400 ng/mL caffeine at 220 nm. [c] Limit of detection based on S/N = 3. [d] Calculated as $N = 16 (t/w)^2$. [e] Calculated at 5% peak height.

1.49% for aspirin, 0.08-1.17% and 0.06-1.09% for caffeine, and 0.09-1.55% and 0.07-2.10% for butalbital, respectively.

Accuracy of intra and inter-day were in the 0.70-1.27% and 0.20-1.13% for aspirin, 0.05-0.06% and 0.004-0.09% for caffeine, and 0.02-0.09 and 0.02-0.05% for butalbital, respectively. The limits of detection for aspirin, caffeine, and butalbital were 5, 5, and 10 ng/mL, respectively, based on a signal noise ratio of 3 and a 50 μL injection. For mixture 2, intra- and inter-day RSD values were 0.06-2.64% and 2.18-4.19% for acetaminophen, 2.24-4.76% and 4.09-4.12% for caffeine, and 1.65-3.94% and 3.33-3.40% for butalbital, respectively.

Accuracy of intra and inter-day were in the 1.13-2.86% and 0.08-0.10% for acetaminophen, 1.12-4.62% and 0.04-2.75% for caffeine, and 0.34-3.48% and 0.02-2.30% for butalbital, respectively. The limits of detection for acetaminophen, caffeine, and butalbital were 20, 20, and 35 ng/mL, respectively, based on a signal noise ratio of 3 and a 10 μL injection. Application of the developed methods to commercial tablet dosage forms is shown in Table 2.

Table 2

Determination of Aspirin, Caffeine, and Butalbital (Mixture 1) in a Commercial Tablet Dosage Form[a] and Acetaminophen, Caffeine, and Butalbital (Mixture 2) in a Commercial Tablet Dosage Form[b]

Analyte	Labeled Amount in Tablet, mg	Amount Found mg[c]	RSD%	% Recovery
Mixture 1				
Aspirin	325	323 ± 20.65	6.38	99.58
Caffeine	40	39.23 ± 2.39	6.09	98.09
Butalbital	50	49.42 ± 2.86	5.80	98.84
Mixture 2				
Acetaminophen	325	320.4 ± 18.56	5.79	98.60
Caffeine	40	39.72 ± 1.95	4.91	99.29
Butalbital	50	49.07 ± 2.76	5.62	98.14

[a] Fiorinal™, Lot no. 779W 6330, Novartis, East Hanover, NJ. [b] Fioriset™, Lot No. 134W6142, Novartis, East Hanover, NJ. [c] Mean ± Std. Deviation based on n = 3.

Based on the data received, the NPS methods for Mixtures 1 and 2 are suitable replacements for current USP 23 methods since they provided excellent peak separation and yet preserved peak resolution.

There was a concern that column wetting with only 2-3% organic phase in the mobile phase would reduce plate numbers and cause fluctuations in retention times due to difficulty in controlling the exact volume of organic modifier. The day-to-day retention time fluctuations were overcome with the use of fresh mobile phase prepared daily.

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