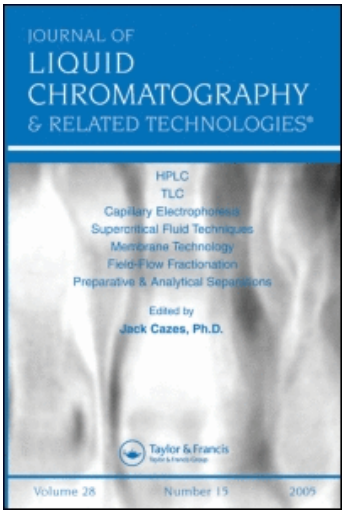

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ANALYSIS OF AN ANALGESIC MIXTURE UTILIZING A NONPOROUS OCTADECYLSILANE STATIONARY PHASE FOR REVERSED-PHASE FAST HPLC

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ABSTRACT

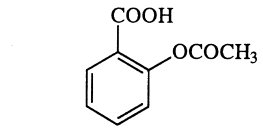
A High Performance Liquid Chromatography (HPLC) method utilizing nonporous silica particle technology has been developed for the assay of an analgesic mixture containing aspirin, caffeine, and codeine. The separation and quantitation of the mixture was achieved on a column packed with nonporous silica particles chemically bonded with octadecylsilane (3.0 μm particles) using a mobile phase of 95:5 v/v 0.01 M phosphate buffer (pH 3.0)-methanol at a flow rate of 600 μL/min with UV detection at 216 nm. The separation was achieved within 3 min. The method showed linearity for aspirin in the range 40 - 242.5 μg/mL, and for caffeine and codeine in the range 7.5 - 45 μg/mL. Inter- and intra day precision ranged from 0.12-1.95% for aspirin, 0.07-1.69% for caffeine, and 0.06-1.69% for codeine. The limits of detection for the analytes were 157 ng/mL for aspirin, 28 ng/mL for caffeine, and 30 ng/mL for codeine based on a signal-to-noise ratio (S/N) of 3 and a 10 μL injection.

INTRODUCTION

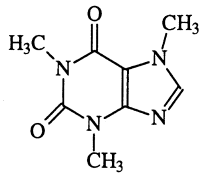
The need for newer, faster high performance liquid chromatography (HPLC) methods has become an important issue in the pharmaceutical industry, as well as with contract laboratories. In the age of smaller budgets and increased workloads in analytical laboratories, the greater the throughput of an HPLC assay, the larger the number of samples that can be analyzed, resulting in developmental goals being met on time at lower cost. Most modern HPLC systems are equipped with traditional stainless steel columns, such as those 150 to 300 mm in length, containing porous silica particles ranging from 5-10 μm in diameter. However, in recent years, a new type of stationary phase has been developed. Jenke[1] and Lee et al[2] have investigated the use of packed HPLC columns utilizing a nonporous silica (NPS) particle, with a diameter of 1.5 μm, bonded with octadecylsilane (ODS). Both investigators provided very useful theoretical information concerning the use of this relatively new particle technology. Van Deemter plots comparing reduced plate height compared to reduced mobile phase velocities were relatively flat compared to conventional HPLC columns in both papers. This was attributed to stagnant mobile phase mass transfer effects that were apparently minimized by the nonporous silica packing, resulting in a reduction of plate height and a more efficient HPLC column and better separation. Nonporous silica particles also tend to have more uniform particle shape and size compared to most 5 μm particles,[3,4] contributing to a more efficient column. However, in utilizing nonporous silica columns, the retention factor is closely related to the organic modifier fraction[1]. As a result, small changes in the organic fraction can result in fairly significant changes in retention. There is also the need to reduce extra column effects with nonporous silica columns, such as dissolving the analyte in a solvent closely matched to the mobile phase, lest peak shape (increase of tailing factors) suffer during analysis, as well as the need to minimize dead volumes in the entire HPLC system.

Many HPLC methods have been demonstrated for multi-component analgesic mixtures containing aspirin, caffeine, and codeine.[5,6] However, these methods typically take from several min up to almost 45 min. The development of an assay to analyze multi-component mixtures within 3 min would result in higher throughput, as well as reduction of solvent usage for a specified number of samples compared to conventional HPLC assays, resulting in lower costs.

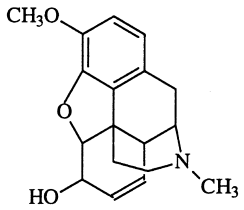
In this paper, an isocratic HPLC method has been developed that will simultaneously quantitate aspirin, caffeine, and codeine utilizing a nonporous ODS column in a single injection in $\leq$3 min at ambient temperature. The sensitivities of the method extend to the ng/mL range for all three components. A comparison HPLC run was made utilizing the official USP compendial method.



Aspirin



Caffeine



Codeine

Figure 1. Structural formulae of aspirin, caffeine, and codeine.

EXPERIMENTAL

Reagents and Chemicals

The structure formulae of the components studied are shown in Figure 1. Aspirin, caffeine, and codeine phosphate were obtained from Sigma Chemical Co. (St. Louis, MO 63178).

Methanol (J.T. Baker, Phillipsburg, NJ 08865) was HPLC grade and water was purified by a cartridge system (Continental Water Systems, Roswell, GA 30076). Monobasic potassium phosphate was J. T. Baker analyzed reagent (Phillipsburg, NJ 08865).

Instrumentation

The chromatographic separations were performed on an HPLC system consisting of a Micra ODS-IIE (monomeric and endcapped) column (100 x 4.6 mm i.d.) containing 3.0 μ nonporous silica (NPS) microspheres chemically bonded with octadecylsilane obtained from Micra Scientific (Northbrook, IL 60062), an Alcott Model 708 autosampler (Norcross, GA 30093) equipped with a 10 μL loop, a Micromeritics Model 760 HPLC Pump (Norcross, GA 30093), a Kratos Spectroflow 757 Absorbance Detector (Ramsey, NJ 07446), and a Hewlett Packard Model 3392A Integrator (Atlanta, GA 30339). The mobile phase consisted of 95:5 v/v 0.01 M aqueous monobasic potassium phosphate, pH 3.0 (adjusted with phosphoric acid)-methanol, with a flow rate of 600 μL/min.

A comparison HPLC run was made utilizing the official USP compendial method for the analgesic mixture on an Alltech silica column (150 x 4.6 mm i.d.) containing 5 μm bare silica particles (Deerfield, IL 60015). The mobile phase consisted of 50:50 v/v 0.01 M aqueous monobasic potassium phosphate, pH 3.0 (adjusted with phosphoric acid)-methanol with a flow rate at 500 μLmin.

Both mobile phases were filtered through a 0.45 μm Nylon-66 filter (MSI, Westborough, MA 01581) and degassed. The detector was set at 216 nm to analyze all three components in the mixture with both columns.

Preparation of Standard Solutions

Individual stock solutions were prepared for all three analytes. Aspirin stock solutions were prepared daily by weighing 20 mg of aspirin and dissolving in 20 mL of 85:15 v/v deionized water-methanol. Caffeine and codeine stock solutions were prepared weekly by weighing 4 mg of each and dissolving in 20 mL of 85:15 v/v deionized water-methanol. Mixtures of the three components were prepared with appropriate dilutions of the three stock solutions to obtain solutions containing 40, 80, 162.5, and 242.5 μg/mL of aspirin and 7.5, 15, 30, and 45 μg/mL each of caffeine and codeine, with dilutions accomplished with mobile phase added to volume.

Four point calibration curves were constructed for each analyte in the mixture. Spiked samples containing 60 and 200 μg/mL for aspirin and 10 and 35 μg/mL each for caffeine and codeine were prepared to obtain accuracy and precision of the method. Quantitation was based on linear regression analysis of analyte peak area versus analyte concentration in μg/mL.

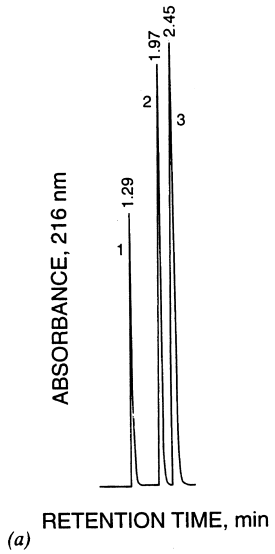


Figure 2a. HPLC chromatogram of codeine (1), aspirin (2), and caffeine (3) on a nonporous ODS column (100 x 4.6 mm i.d.). See Experimental Section for details.

RESULTS AND DISCUSSION

The objective of this study was to develop a fast HPLC method utilizing nonporous silica technology to analyze a three component analgesic mixture. There are several HPLC methods in the literature that discuss the analysis of analgesic mixtures, but none describing the separation and quantitation utilizing nonporous octadecylsilane bound silica as the stationary phase.

In the development of this method, several NPS columns were examined with 33 and 100 mm lengths available commercially. The 33 mm nonporous ODS columns contained 1.5 μm diameter particles, whereas the 100 mm nonporous ODS columns contained 3.0 μm particles. It was determined that a 100 x 4.6 mm i.d. monomeric nonporous ODS column with end-capping provided the best separation and peak shapes, with a short retention time. The reasons for its selection are its larger capacity, and subsequent baseline resolution ($R_s \geq 1.5$) of the three components in the mixture, better peak shapes for all components (lower tailing factors), as well as a reduction in back pressure. Various mobile phases were also tested, ranging from 5 to 30% methanol content.

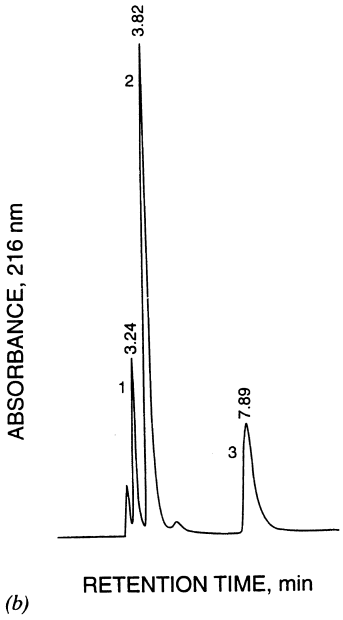


Figure 2b. HPLC chromatogram of codeine (1), aspirin (2), and caffeine (3) on a bare silica column (150 x 4.6 mm i.d.). See Experimental Section for details.

It was determined that 5% methanol in the mobile phase provided the optimized separation. However, it was also observed that changes of ± 2-3% methanol in the mobile phase lead to changes of the retention factors of analytes, resulting in the loss of resolution of some of the peaks, similar to observations with alkyl phthalates cited by Jenke.[1] It was also observed that the nonporous ODS column was less sensitive to changes in flow rate compared to a conventional bare silica column, although the changes were more pronounced on a 3.0 μm compared to a 1.5 μm NPS ODS column. A typical chromatogram of the separation of aspirin, caffeine, and codeine on the nonporous octadecylsilane column can be seen in Figure 2a.

A comparison HPLC run was made on a conventional bare silica column using the official USP compendial method for aspirin, caffeine, and codeine. The separation of the three components is shown in Figure 2b. Analytical figures of merit for both columns are listed in Table 1. As shown in the chromatograms, the run using the USP compendial method resulted in a total run time of ~ 8.5 min, whereas the run time on the nonporous ODS column was ~ 3.0 min.

Table 1

Analytical Figures of Merit for Aspirin, Caffeine, and Codeine

Column	Analyte	k	N[a]	T[b]	R_s	α
Nonporous ODS	Codeine	0.18	6.23	1.0	11.0	7.4
	Aspirin	1.33	2443	1.0	3.8	1.3
	Caffeine	1.78	2864	1.2		
Bare Silica	Codeine	1.00	3462	1.8	3.9	1.4
	Aspirin	1.36	2852	1.6	13.6	2.8
	Caffeine	3.80	1948	1.9		

[a] Calculated as $5.54 (t_r/t_w)^2$.
[b] Calculated at 5% peak height.

Table 2

Inter- and Intraday Linearity Data

Analyte	r^2(Day 1)	r^2(Day 2)	r^3(Day 3)
Aspirin[a]	0.9996 (Run 1) 0.9991 (Run 2)	0.9991	0.9992
Caffeine[b]	0.9996 (Run 1) 0.9994 (run 2)	0.9996	0.9999
Codeine[c]	0.9992 (Run 1) 0.9993 (Run 2)	0.9990	0.9999

[a] Range examined from 40 - 242.5 µg/mL Aspirin (based on n=16 for each curve).
[b] Range examined from 7.5 - 45µg/mL Caffeine (based on n=16 for each curve).
[c] Range examined from 7.5 - 45µg/mL Codeine (based on n=16 for each curve).

Table 3

Accuracy and Precision Data with Spiked Samples

Analyte	Conc. Added (μg/mL)	Conc. Found (μg/mL)[a]	Percent Error	% RSD
Aspirin	60	59.1 ± 0.11	1.63	0.18
	200	197.4 ± 2.50	1.32	1.26
Caffeine	10	10.24 ± 0.09	2.47	0.87
	35	35.8 ± 0.10	2.54	0.28
Codeine	10	9.9 ± 0.03	0.53	0.28
	35	34.8 ± 0.01	0.54	0.54

[a] Based on n = 4.

To assure that salicylic acid, the hydrolysis product of aspirin, did not interfere in the assay using the nonporous ODS column, a mixture of the three components was allowed to sit at ambient temperature for 24 hours, whereupon it was determined that salicylic acid did not interfere with any of the peaks. Limits of detection were also determined on the NPS column at a signal-to-noise ratio of 3 for aspirin (157 ng/mL), caffeine (28 ng/mL), and codeine (30 ng/mL).

Inter- and intra day linearity of the assay are shown in Table 2. The correlation from day to day was very good for each component of the mixture. Inter- and intra day precision ranged from 0.12-1.95% for aspirin, 0.07-1.69% for caffeine, and 0.06-1.69% for codeine. Accuracy and precision were evaluated for each component in the mixture using spiked samples. The data shown in Table 3 indicates that the method gives acceptable accuracy and precision for each analyte in the mixture.

In summary, a nonporous ODS column with a 95:5 v/v aqueous phosphate buffer (pH 3.0)-methanol mobile phase was developed for the analysis of aspirin, caffeine, and codeine in an analgesic mixture. The run times were $\leq$3.0 min, solvent usage was reduced, and the column showed at least equivalent efficiency compared to a conventional bare silica column used in the official USP compendial method. Reduction of dead volumes and small injection volumes must be adhered to in order to achieve optimal results, as well as keeping the amount of organic modifier in the mobile phase within 1% of the specified amount.

Nonporous ODS columns show tremendous potential to replace larger conventional columns in many applications in the analytical laboratory as demands for higher throughput continue to increase.

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