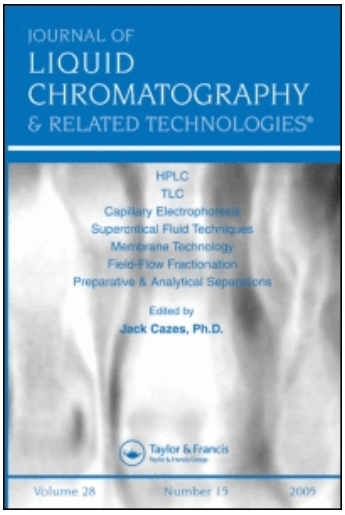

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Development and Validation of an HPTLC-Densitometry Method for Assay of Caffeine and Acetaminophen in Multicomponent Extra Strength Analgesic Tablets

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ABSTRACT

A quantitative method using silica gel HPTLC plates with fluorescent indicator, automated sample application, and UV absorption densitometry has been developed for the determination of caffeine and acetaminophen in pharmaceutical preparations. Multicomponent analgesic tablets containing caffeine, acetaminophen, and acetylsalicylic acid as the active ingredients were analyzed to test the applicability of the new method. Precision for the caffeine analysis was evaluated by replicate analysis of samples and accuracy by analysis of two spiked blank samples containing inactive ingredients in common with the multicomponent analgesic tablets. The amount of caffeine in the tablets analyzed ranged from 96 to 115% of the label value. Precision was 1.19% relative standard

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deviation, and the errors from the two spiked blank analyses averaged 1.90% compared with fortification levels. The limit of detection was 0.200 μg of caffeine. Precision for acetaminophen analysis was evaluated also by replicate analysis of samples, and accuracy was measured by standard addition analysis. The amount of acetaminophen in the tablets analyzed ranged from 97 to 110% of the label value. Precision ranged from 0.949 to 7.56% difference for duplicate samples. For the standard addition analysis, the error was 1.03% compared with fortification levels. The limit of detection was 0.0800 μg of acetaminophen.

Key Words: Caffeine; Acetaminophen; High-performance thin layer chromatography; Densitometry; Analgesics.

INTRODUCTION

In previous papers, new instrumental, quantitative high performance thin layer chromatography (HPTLC)–densitometry methods were reported for determination of caffeine in beverages[1] and pharmaceutical alertness tablets and caplets.[2] The purpose of this research was to extend the previous HPTLC methodology for caffeine analysis to multicomponent tablets that contain acetaminophen and acetylsalicylic acid. Computerized searches of the literature using Chemical Abstracts, the ISI Web of Science, Medline, and Camag Bibliography Service (CBS) found densitometric HPTLC methods for determination of caffeine with chlorphenoxamine hydrochloride and 8-chlorotheophylline[3] and acetaminophen with orphenadrine citrate[4] in pharmaceutical dosage forms, as well as methods for caffeine in clinical and veterinary samples.[5] However, no methods were found for analysis of caffeine in products containing acetaminophen and acetylsalicylic acid. The new method described below provides simple, inexpensive, and rapid assay of caffeine, as well as acetaminophen, in tablets containing these three ingredients. It was validated for factors such as accuracy, precision, linearity, and sensitivity, and it was found to be suitable for use in routine analysis in pharmaceutical industry quality control and regulatory agency compliance laboratories.

EXPERIMENTAL

Preparation of Standard Solutions

A caffeine (3,7-dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione; #C-0750, Sigma, St Louis, MO; CAS registry no. 58-08-2) stock standard solution

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(10.0 mg mL^{-1}) was prepared in methanol. Magnetic stirring for 30 min was required to complete dissolution. A caffeine TLC standard was prepared by 1 : 100 dilution with methanol to a concentration of 0.100 mg mL^{-1}.

An acetaminophen (4-acetaminophenol; #A-7085, Sigma; CAS registry no. 103-90-2) stock standard solution (1.00 mg mL^{-1}) was prepared in methanol and diluted 1 : 10 with methanol to prepare a 0.0200 mg mL^{-1} TLC standard solution.

Preparation of Sample Solutions

One national manufacturer's brand and one generic brand of extra strength analgesic tablets with label specifications of 65 mg caffeine, 250 mg acetaminophen, and 250 mg acetylsalicylic acid were purchased from a local pharmacy. After experimenting with different sample preparations such as stirring, stirring with sonication, and removal of inert ingredients by filtration or settling, the following was found to be the most accurate method. Sample stock solutions were prepared by grinding one tablet to a fine powder, using a mortar and pestle, and completely transferring into a 100 mL volumetric flask by washing with about 70 mL of methanol. The solution was stirred magnetically for 30 min, and the stir bar was removed with a magnetic rod. The solution was diluted to the line with methanol and mixed by shaking, and the solution was allowed to stand for 1 h, so that the undissolved excipients settled to the bottom of the flask. The caffeine sample TLC test solution was prepared by a 1 : 10 dilution of the clear sample stock solution by mixing 100 μL of caffeine sample stock solution with 900 μL of methanol. Digital Drummond (Broomall, PA) microdispensers (10, 100, and 1000 μL) were used to measure volumes for preparation of various solutions throughout this research. The theoretical concentration of the caffeine test solution was 0.0650 mg mL^{-1}, based on the label value. For acetaminophen analysis, the TLC sample solution was prepared by two 1 : 10 dilutions of the sample stock solution. The theoretical acetaminophen concentration of the test solution was 0.0250 mg mL^{-1}.

High Performance Thin-Layer Chromatography Analysis

Different brands and types of silica gel TLC and HPTLC plates were tested, and 20 × 10 cm high-performance silica gel 60F_{254} GLP plates (No. 5613/6, EM Science, Gibbstown, NJ, an affiliate of Merck, Darmstadt, Germany) were found to give the best results and were used for all analyses. Sample and standard solutions for caffeine and acetaminophen determinations were applied with a Camag (Wilmington, NC) Linomat IV automated spray-on

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band applicator having a 100 μL syringe, and operated with the following settings: band length 6 mm, application rate 4 s μL^{-1}, table speed 10 mm s^{-1}, distance between bands 4 mm, distance from the plate edge 7 mm, and distance from the bottom of the plate 1.5 cm. For caffeine analysis, the volumes applied were 2.00 μL, duplicate 4.00 μL, and 8.00 μL of the TLC standard and duplicate 6.00 μL aliquots of the caffeine sample solutions (0.390 μg theoretical caffeine content). For acetaminophen analysis, the volumes applied were 4.00 μL, duplicate 8.00 μL, and 16.0 μL of the TLC standard and duplicate 8.00 μL aliquots of the acetaminophen sample solutions (0.200 μg theoretical acetaminophen content).

Plates were developed 6 cm beyond the origin, with ethyl acetate–glacial acetic acid (95 : 5) in a Camag HPTLC twin-trough chamber containing a saturation pad (Analtech, Newark, DE). The development time was 15 min.

After development, the plates were dried in a fumehood on a TLC plate heater (Camag) for 5 min. The sample and standard zones were then quantified by use of a Camag TLC Scanner II at a wavelength of 254 nm with the deuterium source, slit length 4, slit width 4, and scanning rate 4.0 mm s^{-1}. The CATS-3 software controlling the densitometer produced a linear regression calibration curve relating the standard zone weights (for caffeine, 0.200–0.800 μg; for acetaminophen, 0.0800–0.320 μg) to their scan areas. Percent recovery was calculated for each sample analysis by comparing the theoretical weight with the mean experimental weight obtained from the duplicate sample zones.

The accuracy of the caffeine analysis was validated by two spiking experiments. An allergy sinus headache caplet containing diphenhydramine (12.5 mg), pseudoephedrine hydrochloride (30 mg) and acetaminophen (500 mg) as active ingredients and an extra-strength gelcap containing acetaminophen (500 mg) as the active ingredient were used as blanks. These were appropriate blanks because they contained fourteen of the inactive ingredients present in the two brands of tablets analyzed (Table 1). The additional inactive ingredients present in the blank solutions might be present in other extra strength analgesic brands that were not analyzed. Preliminary experiments showed that the diphenhydramine, pseudoephedrine, and acetaminophen zones in the blanks were widely separated from the caffeine zones. To prepare the blank stock solutions, an allergy sinus tablet and acetaminophen gelcap were prepared as described above, but caffeine stock standard solution (6.50 mL) was added to the 100-mL volumetric flask before dilution to the line, to simulate a caffeine tablet containing exactly the label amount. Unspiked blanks were also prepared. A 1 : 10 dilution was done to prepare the test spiked and unspiked blank solutions, as described above. The caffeine standard, the unspiked blank test solution, and the spiked blank solution were spotted on one plate and analyzed as described above. Percent

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Table 1. Inactive ingredients in the blanks.

Ingredients in the blanks and in the two tablet brands analyzed	Additional inactive ingredients in the blanks
Carnauba wax	Silicon dioxide
Povidone	Fractionated coconut oil
Corn starch	Disodium EDTA
Propylene glycol	Red #40
Crospovidone	Edible ink
Sodium saccharin	Yellow #10
FD&C Blue No.1	Gelatin
Stearic acid	Hydrogenated starch hydrolysate
Hydroxypropyl methylcellulose	Glycerin
Titanium dioxide	Magnesium stearate
Microcrystalline cellulose	Triacetin
Croscarmellose sodium	Polyethylene glycol
Hydroxypropyl cellulose	Pregelatinized starch
Polysorbate 20	Sodium starch glycolate
	Zinc stearate

recovery was calculated by comparing the analytical result for the spiked blank solution to the theoretical value based on the weight of the caffeine added.

The accuracy of the acetaminophen analysis was validated by standard addition analysis. A 250-mg tablet stock solution was prepared according to the procedure described above, and 8.00-μL was mixed with 992-μL of methanol in a 6-mL screw cap vial to prepare a TLC test solution with a theoretical concentration of $0.0200\,\mathrm{mg\,mL^{-1}}$. A 300-μL aliquot of this solution was mixed with 6.00-μL of the acetaminophen standard stock solution to double the acetaminophen concentration based on the label value. The original and fortified solutions were analyzed on the same plate by application of duplicate 8.00- and 4.00-μL volumes, respectively, and the four standards as described above for acetaminophen. The difference between the mean experimental weights and the added weight was calculated to determine the accuracy of the method.

Precision for caffeine was validated by spotting six 6.00-μL aliquots of the test solution and standard solutions on a single plate. The plate was developed and scanned, and the percent relative standard deviation of the experimental weights was calculated. As an additional measure of precision, the percent difference between the areas of the duplicate caffeine and acetaminophen zones applied in each analysis was calculated.

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RESULTS AND DISCUSSION

In preliminary sample preparation studies, it was found that methanol quickly dissolved both the caffeine and acetaminophen standards and completely extracted both ingredients from unground tablets treated by stirring. Sonication was not used to aid extraction because it gave low results when samples were analyzed. Insoluble sample excipients settled to the bottom of the volumetric flask after one hour, so a filtration step was not needed to produce a clear sample test solutions for spotting on HPTLC plates.

A number of brands and types of TLC and HPTLC plates were tested for efficiency and selectivity of separations and accuracy and precision of analyses, and Merck 20×10 cm high-performance silica gel $60F_{254}$ GLP plates were found to produce the best results. Other plates gave low percent recoveries and precision, as well as poorer resolution and theoretical plate numbers. Theoretical plate numbers for the Merck plate were 6.1×10^3 for caffeine, 3.5×10^2 for acetaminophen, and 1.8×10^4 for acetylsalicylic acid, and resolution between the acetylsalicylic acid and acetaminophen zones was 4.33. These N and R values were calculated from measurements of the zones made directly on the layer, as described by Kowalska et al.[6]

Development on HPTLC silica gel layers containing fluorescent indicator, using ethyl acetate–glacial acetic acid (95 : 5) mobile phase, produced flat, tight, dark bands on a bright green background under 254 nm UV light for both caffeine and acetaminophen. R_f values were 0.24 and 0.55, respectively (Fig. 1). The correlation coefficient (r) values of the calibration plots produced by linear regression of the four standards, for each analysis of both drugs, ranged from 0.992–0.999.

As recommended by The International Committee on Harmonization (ICH) guidelines,[7] a calibration curve was established for caffeine using five analyte concentrations (2.00, 3.00, 4.00, 6.00, and 8.00 μL zone^{-1} of the TLC standard), representing 0.200–0.800 μg of caffeine. The r value of this curve was 0.999. For routine analytical procedures, a three-point calibration curve within this range was used, produced by applying 2.00, 4.00, and 8.00 μL of the caffeine TLC standard on each plate. For acetaminophen, a five point calibration curve used five analyte concentrations (4.00, 6.00, 8.00, 10.0, and 16.0 μL zone^{-1} of the TLC standard), representing 0.0800–0.320 μg of acetaminophen. The r value of this curve was 0.999. The three-point calibration curve was produced by applying 4.00, 8.00, and 16.0 μL of the acetaminophen TLC standard on each plate.

The limit of detection for caffeine was determined by viewing developed plates containing 0.140 to 3.20 μg standard zones under 254-nm UV light. The zone from 2.00-μL of the 0.100-mg mL^{-1} solution (0.200 μg) was barely visible, and the zone from 2.00-μL of a 0.700-mg mL^{-1} solution (0.140 μg)

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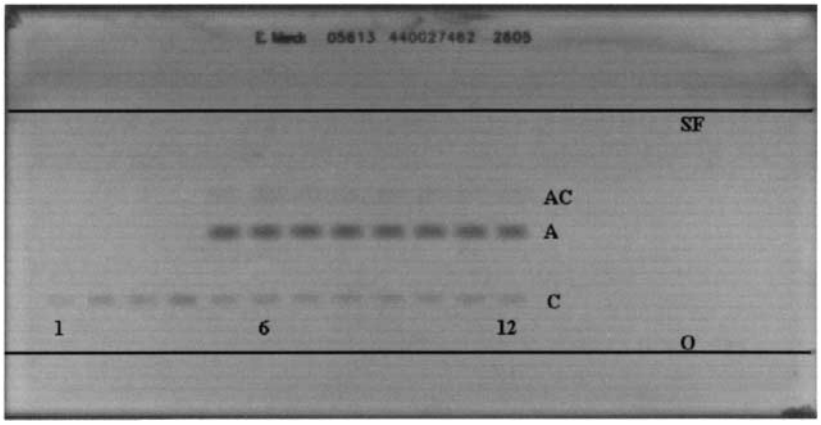

Figure 1. Chromatograms obtained from analysis of caffeine in multicomponent analgesic tablets by use of the HPTLC-densitometry method described. The plate was photographed under 254-nm UV light with a Camag VideoStore image documentation system. SF, mobile phase front; O, origin; lanes 1–4, caffeine (C) standard zones; lanes 5 and 6, 7 and 8, 9 and 10, and 11 and 12 are duplicate zones of tablets 1–4, respectively. These samples all contain caffeine (C), acetaminophen (A), and acetylsalicylic acid (AC).

was not visible. For acetaminophne, the limit of detection was determined by spotting 0.0400–0.200 μg standard zones. The zone from 4.00-μL of the 0.0200-mg mL^{-1} solution (0.0800 μg) was barely visible, and the zone from 2.00-μL of the 0.0200-mg mL^{-1} solution (0.0400 μg) was not visible. From these results, the limits of detection and quantification were taken as 0.200 μg for caffeine and 0.0800 μg for acetaminophen, and these amounts were the lowest weights used for the respective calibration curves as described above.

Five different caffeine tablets and six acetaminophen tablets were analyzed by the described procedure with $n = 2$. The recoveries compared to the label value are shown in Table 2. It can be seen that two of the results for caffeine were not within the ±10% specification range stipulated in the USP[8] for acetaminophen and caffeine tablets.

A sixth caffeine tablet was analyzed six times ($n = 6$) on one plate, and the average recovery was 101.8 ± 1.19% (mean ± relative standard deviation). As an additional measure of precision, differences between scan areas of duplicate sample aliquots spotted in caffeine analyses ranged from 0.193–1.79%, with an average of 0.945%. For acetaminophen analyses, differences for duplicate sample areas ranged from 0.949–7.56%, with an average of 3.59%.

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Table 2. Recoveries (%) from tablets relative to the label values.

Sample	Caffeine	Acetaminophen
Tablet 1	96.2	97.0
Tablet 2	94.9	102.0
Tablet 3	115.4	110.0
Tablet 4	97.4	102.5
Tablet 5	111.5	107.0
Tablet 6		104.5
Tablet 7		101.3

Note: Recoveries are average values for duplicate spotted samples ($n = 2$).

The accuracy of the caffeine analysis was validated by two spiking experiments. The active ingredients in the blanks did not interfere with the caffeine zones ($R_f = 0.24$) because diphenhydramine, pseudoephedrine hydrochloride, and acetaminophen all had R_f values of 0.58 after development with the mobile phase. Analyses resulted in recoveries of 103.3% (3.3% error) for the extra-strength gelcap caffeine blank and 100.5% (0.5% error) for the allergy sinus headache caplet caffeine blank. No zone occurred at the R_f value of caffeine in unspiked blank chromatograms, and no area counts were obtained when this region of the layer was scanned.

For the validation of accuracy of the acetaminophen analysis, standard addition was carried out for tablet number 7 (Table 2), in which unspiked and spiked solutions were analyzed on the same plate. The recovery of the added weight was 101.3% (1.03% error).

The third active ingredient in the analyzed tablets, acetylsalicylic acid ($R_f = 0.71$), could not be quantified because the scan areas of its standard and sample zones were not reliable measurements of their weights. The resolution between the acetaminophen and acetylsalicylic acid zones was adequate ($R = 4.33$), but the acetylsalicylic acid zones were too close to the mobile phase front, which caused their scan peaks to tail and not return to baseline. In order to determine, simultaneously, all three active tablet components, a stationary phase-mobile phase system that lowered the R_f value of acetylsalicylic acid but maintained adequate resolution among all of the compounds would be necessary. Such a system was not found in this research.

For successful validation of pharmaceutical assay procedures, the ICH requires accuracy (recovery) and precision (RSD) of 95–105% and 3% or less, respectively.[7] The data presented above show that the new HPTLC method meets these standards, as well as those suggested by the ICH for

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other validation parameters, such as linearity and limits of detection and quantification.

A computerized literature search did not locate a previously published high performance column liquid chromatography (HPLC) method for determination of caffeine and acetaminophen in multicomponent analgesic tablets of the type we analyzed, so a direct comparison cannot be made. However, a method was published recently for the HPLC determination of these compounds in other pharmaceutical samples,[9] in which a C-18 bonded phase column, water, and then acetonitrile–water (75 : 25) eluents, and UV detection at 285 and 240 nm were used. Recovery and RSD values for caffeine ranged from 98–102.4% and 0.6–1% for caffeine, respectively, and 95–100% and 0.6–2% for acetaminophen, respectively, for HPLC, which are no better than the results we obtained with HPTLC. Our results are also at least as good as those regularly reported in the literature for HPLC and HPTLC of other drugs, in a variety of pharmaceutical dosage forms.[10]

Sample treatment for HPTLC is very simple, and no interference from excipients was found. The time for analysis on a per-sample basis is low, because up to seven samples can be analyzed in duplicate with the four standards needed to prepare the calibration curve in a single run on the same plate, rather than performing sequential injection of the samples and standards in HPLC. This simultaneous chromatography of samples and standards, under identical conditions, leads to the excellent analytical accuracy and precision values that were obtained. The method will be useful for quality control of tablets by manufacturers and survey analysis by regulatory agencies.

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