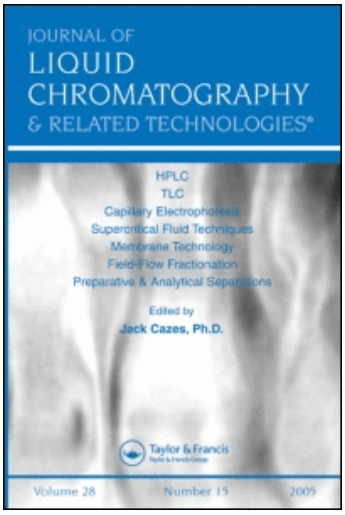

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DETERMINATION OF THE SLEEP AID INGREDIENTS DIPHENHYDRAMINE HYDROCHLORIDE AND DOXYLAMINE SUCCINATE IN PHARMACEUTICAL PRODUCTS BY QUANTITATIVE HPTLC

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ABSTRACT

A quantitative method, using high performance thin layer chromatography (HPTLC) with automated sample application and UV-absorption scanning densitometry, was developed for the determination of diphenhydramine hydrochloride and doxylamine succinate in pharmaceutical sleep aid products. Separation was performed on high performance silica gel plates, and the analytes were detected as fluorescence-quenched zones under 254 nm UV light. Three pharmaceutical products containing diphenhydramine hydrochloride as an active ingredient, and three containing doxylamine succinate as an active ingredient, were analyzed to test the applicability of the method. Precision was validated by replicate analyses of samples and accuracy by analysis of spiked samples.

The percent diphenhydramine hydrochloride in the tested pharmaceutical samples ranged from 97.9% to 113% compared to label values; precision ranged from 0.69% to 2.36% relative standard deviation, and the errors in standard addition experiments used to test accuracy varied between 0.00% and

0.26% compared to fortification levels. For doxylamine succinate, samples assayed at 93.9-107% relative to the label declaration; precision ranged from 0.75% to 2.13%, and errors from standard addition and blank spike analyses were 0.38% and 1.59%, respectively.

INTRODUCTION

Diphenhydramine hydrochloride (DPH) and doxylamine succinate (DS) are widely used active ingredients in sleep aid pharmaceuticals and night-time antihistamine-decongestant medicines. A quantitative high performance thin layer chromatography (HPTLC) method involving automated sample application and scanning densitometry was developed for DPH in antihistamine tablets, gelcaps, and capsules.[1] That method is extended in this paper to the analysis of sleep formula tablets containing DPH as the only active ingredient and to night-time analgesic caplets containing aspirin (AS) or acetaminophen (AM) plus DPH. In addition, the HPTLC method is applied to the determination of DS as the only active ingredient in two different brands of sleep aid tablets and in night-time cold and flu liquid caps also containing dextromethorphan hydrobromide (DM), pseudoephedrine hydrochloride (PE), and (AM).

Earlier HPLC and TLC methods for DPH were reviewed in our previous paper.[1] The current method for assay of DS in drug raw material involves acid-base titration, while DS tablets are assayed by ultraviolet (UV) spectrometry.[2] No quantitative HPTLC method for determination of DS in pharmaceutical tablets or liquid caps was found in the literature. The new method described is selective, accurate, and precise, and it is cost effective and rapid on a per-sample basis.

EXPERIMENTAL

Preparation of Standard Solutions

The standards used to make the stock solutions were DPH reference standard (N-[2-diphenylmethoxyethyl]-N,N-dimethylamine hydrochloride, CAS #: 147-24-0, Sigma, St. Louis, MO) and DS reference standard (doxylamine succinate salt, CAS #: 562-10-7, Sigma). These standards were dissolved in absolute ethanol to make a 100.0 mg/mL standard stock solution of DPH and a 10.0 mg mL^{-1} stock solution of DS. The TLC standards (1.00 mg/mL) for both DPH and DS were prepared by appropriate dilution of their respective stock solutions with ethanol.

Preparation of Sample Solutions

Six pharmaceutical samples described in Table 1, including tablets, caplets, and gelatin caplets containing DPH, and tablets and liquid caps containing DS, were purchased at a drug store. Tablets and caplets were ground into a fine powder by use of a mortar and pestle, and gelatin caplets and were cut open with a razor before being ground. The resulting powder was transferred completely through a funnel into a 100 mL volumetric flask using ca. 90 mL of ethanol. The liquid caps were cut open with a razor, and the gel was rinsed completely into a 200 mL volumetric flask using ca. 180 mL of ethanol. The solutions were magnetically stirred for 1 hr, diluted to volume, and shaken. All solutions, except for those containing the liquid caps, were allowed to sit for ca. 2 hr so that excipients would settle to the bottom of the flask and not be applied to the layer.

HPTLC Analysis

Analyses were performed on 20 x 10 cm silica gel 60 F_{254} GLP plates (No. 5613/3, EM Separations Technology, Gibbstown, NJ). Sample and standard solutions were applied by means of a Camag (Wilmington, NC) Linomat IV automated spray-on band applicator equipped with a 100 μL syringe using parameters specified in a previous paper.[1] For the analysis of Samples 1 through 3 containing DPH, 2.00 μL and 8.00 μL and duplicate 4.00 μL aliquots of the 1.00 mg/mL standard and duplicate 16.0 μL aliquots of sample were spotted on the plate. For analysis of Samples 4 through 6 containing DS, the aliquots of the 1.00 mg/mL DS standard were the same as those for the DPH standard, but duplicate 2.00 μL aliquots of Samples 4 and 5 and duplicate 8.00 μL aliquots of Sample 6 were spotted on the plate instead of duplicate 16.0 μL sample aliquots.

Plates were developed for a distance of ca. 6 cm beyond the origin in a vapor-equilibrated, paper-lined Camag twin-trough HPTLC chamber using ethyl acetate-methanol-concentrated ammonium hydroxide (85:10:5) as the mobile phase for plates containing Samples 1 through 5. The development time was 15-18 min, after which the plates were allowed to air dry. For plates on which Sample 6 was spotted, butanol-water-glacial acetic acid (70:20:10) was used as the mobile phase, and the development time was ca. 1.6 hr. Standard and sample zones were quantified by scanning at 260 nm for DPH and 265 nm for DS by use of a Camag TLC Scanner II with the deuterium source, slit dimension settings of length 4 and width 4, and a scanning rate of 4 mm/sec. The wavelengths used were those found to give maximum absorption by measurement of in situ spectra of standard zones with the spectral mode of the densitometer. The CATS-3 software polynomial regression program furnished a calibration curve relating standard zone weights to their optimized scan areas.

The analyte weights in the sample zones were determined automatically from their areas by interpolation from the calibration curve. For each tablet analysis, percent recovery was determined by comparing the theoretical weight predicted by the label declaration to the mean experimental weight of duplicate sample zones.

To validate the accuracy of the new HPTLC method, spiking experiments were conducted. For Samples 1, 2, 3, and 5, the standard addition method[3] was used in which a sample solution was prepared and analyzed as described above. Based on this analysis, an appropriate amount of TLC standard was added to a 10.0 mL aliquot of the sample solution using a 1000 μL Drummond (Broomall, PA) digital microdispenser, to exactly double the concentration of the analyte.

The spiked sample was analyzed on a second plate by spotting the same standard aliquots and duplicate sample aliquots representing one-half of the volume compared to the pre-analysis. After separation and scanning, the percent difference between the average weights of the analyte found in the spiked and unspiked samples, which were theoretically equal, was calculated to determine the recovery.

Accuracy was determined for Sample 6 using a spiking method based on the addition of the standard solution to a sample of a pharmaceutical product used as a blank.[4] For Sample 6, a non-drowsy cold/flu liquid cap which did not contain DS as an ingredient, but did contain the same amounts of DM, AM, and PE as Sample 6, was used as a blank. Most of the inactive ingredients in the blank (i.e., gelatin, glycerin, polyethylene glycol, povidone, and propylene glycol) were identical to, and others were similar to, the inactive ingredients contained in Sample 6. The liquid cap used as the blank contained guaifenesin (G) as an additional active ingredient.

The spiked blank was prepared by transferring the gel from the liquid cap into a 200 mL volumetric flask, adding ca. 80 mL ethanol, and using a 1000 μL Drummond digital microdispenser to add from the 10.0 mg/mL standard stock solution an amount of DS which was equivalent to the label value of DS in Sample 6. An additional 100 mL of ethanol was added, and the solution was stirred for 1 hr, filled to volume with ethanol, and shaken.

Duplicate 16.0 μL aliquots of the spiked sample and unspiked blank, along with the usual aliquots of the TLC standard, were spotted on a plate, separated, and scanned as described above. The percent difference between the amount of DS determined by the analysis and the amount of DS added was calculated to determine the accuracy of the method.

RESULTS AND DISCUSSION

Sample 6, the liquid gel cap, consisted of a gel enclosed in a plastic capsule. After the capsule was cut open, the gel was squeezed directly into the volumetric flask, and the plastic capsule was also transferred to the flask to minimize loss of any active ingredient. The gelatin caplet, Sample 3, consisted of a powder enclosed in a plastic-like coating. The plastic-like coating was rinsed completely with ethanol but was not transferred to the flask.

When viewed under 254 nm UV light, DPH and DS appeared as flat, dark, compact bands against a green background as a result of the fluorescent phosphor in the layer; their respective R_f values were 0.67 and 0.56 in ethyl acetate-methanol-ammonium hydroxide (85:10:5). The R_f values of AM and AS were 0.48 and 0.07, respectively, and the zones of these compounds did not interfere with analysis of DPH in Samples 2 and 3. Methods for the assay of AM and AS can be found in our previous papers.[5,6] This mobile phase did not provide sufficient separation of the components in Sample 6, but butanol-methanol-water (70:20:10) did. The R_f values in this mobile phase were 0.21, 0.38, 0.47, and 0.78 for DS, DM, PE, and AM, respectively; quantitative methods for the analysis of each of the additional active ingredients have been described previously.[6,7,8]

Polynomial calibration with three standard aliquots[4] proved to be the most suitable approach for producing results with the best precision and accuracy when compared to linear regression[3] or use of area and weight ratios of the sample zones and a single standard.[1] Typical values for the calibration curve for DPH (area vs. standard weights, μg) determined on each plate were a = -99, b = 266, and c= -5 using the equation $y = a + bx + cx^2$. Likewise, typical values for the curve for DS in Samples 4 and 5 were a = -66, b = 3027, and c = -732, while a = 36, b = 2118 and c = -416 were average values for the polynomial calibration curve for Sample 6.

Table 1 summarizes the results of the analyses of samples containing DPH and DS. For DPH (Samples 1 through 3), the recoveries relative to the label declaration based on the analysis of individual dosage forms (n = 1) had an average value of 105%. For Samples 4 through 6 containing DS, the recoveries of samples analyzed one time each varied between 95.0% and 107% based on the label value. As shown in the table, all but one of the tablets containing DPH assayed within the 90-110% specification range in the USP 23/NF[9] for DPH pharmaceuticals, and all of the samples of DS assayed within the range of 92-108% for DS pharmaceuticals specified in the same source.[2] When replicate analyses of individual samples were performed (n = 3 or 4 as shown in Table 1), the relative standard deviation (RSD) values ranged from 0.69% to 2.36% for DPH and from 0.75% to 2.19% for DS.

Table 1

Analysis of Samples

Sample	Sample Type	Label Values Active Ingred.	Recoveries[a]	Mean ± RSD[b]	n	% Error Std. Addn.
1	Tablet	DPH 25 mg	103%, 104%	99.0 ± 0.69%	4	0.00
2	Caplet	DPH 25 mg AS 500 mg	97.9%, 105%	102 ± 2.26%	3	0.26
3	Gelatin Caplet	DPH 25 mg AM 500 mg	109%, 113%	106 ± 2.36%	4	0.00
4	Tablet	DS 25 mg	101%, 105%	103 ± 0.97%	3	---
5	Tablet	DS 25 mg	103%, 107%	101 ± 2.13%	3	0.38
6	Liquid Cap	DS 6.25 mg DM 10 mg PE 30 mg AM 250 mg	95.0%, 95.2%, 97.2%	93.9 ± 0.75%	3	---

[a] Each recovery represents a single analysis of an individual sample realtive to the label declaration.
[b] Mean and RSD are based on replicate analyses of single samples (n = 3 or 4, as indicated).

As a measure of reproducibility, in addition to the RSD for replicate analyses, the percentage differences between scan areas for duplicate sample aliquots were calculated, and the median value was 3.22% for DPH and 1.99% for DS.

The accuracy of the proposed method was validated for the tablets, caplets, and gelatin caplets by the standard addition method, in which the solutions were pre-analyzed, spiked to exactly double the concentration of DPH or DS, and then re-analyzed. Comparison of the unspiked and spiked samples prepared and analyzed as described above yielded errors shown in Table 1, which were all in the range of 0.00% to 0.38%. Accuracy validation for Sample 6 was carried out using a procedure based on the spiking of a blank described above, and comparison of the amount of DS determined by the analysis and the amount of standard DS added yielded an error of 1.59%. The R_f value for G, the additional active ingredient in the blank for Sample 6, was 0.75, so the presence of this zone did not interfere with the scanning of the DS zones. Since the overall composition of the blank liquid cap was similar, but not identical, to that of the sample being analyzed, it was not surprising that the accuracy determined from the spiked blank would be poorer than from the standard addition analyses. However, the error obtained was reasonably low.

It has been demonstrated that the new HPTLC method can achieve recovery as a percentage of label declarations, reproducibility for replicate analyses, and recoveries of spiked samples that are well within the range of values required for use in a pharmaceutical analytical laboratory.[10] In addition, the results demonstrated for accuracy and precision are equivalent or superior to those reported regularly in the literature for HPTLC and HPLC analyses of pharmaceutical drugs.[1,4] Our previous papers describe the overall advantages of quantitative HPTLC relative to spectrometry and HPLC for analysis of pharmaceutical dosage forms.[1,3,7,8]

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