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DEVELOPMENT AND VALIDATION OF A HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHIC METHOD WITH DENSITOMETRIC DETECTION FOR DETERMINATION OF BISACODYL IN PHARMACEUTICAL TABLETS

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SUMMARY

A densitometric method has been developed for determination of the laxative bisacodyl in enteric-coated tablets on channeled, high-performance silica gel layers with pre-adsorbent region and containing a fluorescent indicator. After extraction of the analyte with ethanol the extracts and bisacodyl standards were spotted by use of a Linomat spray-on applicator and the plates developed with ethyl acetate–methanol–glacial acetic acid, 85:10:5, as mobile phase. Quantitative evaluation was performed by measuring the absorbance of the analyte zones at 254 nm in the reflectance mode. The method is shown to have the selectivity, accuracy, precision, and high sample throughput that make it useful for routine analysis of the pharmaceutical preparation in industry quality control and regulatory laboratories. An alternative extraction procedure and mobile phase are suggested for analysis of bisacodyl tablets with different formulations.

INTRODUCTION

Bisacodyl is a laxative that is widely prescribed for relief of constipation and irregularity. It is sold in the form of enteric-coated tablets containing 5 mg of the active ingredient. Most analytical methods published for bisacodyl pharmaceutical dosage forms are based on high-performance column liquid chromatography. For example, the latest USP [1] contains an assay for bisacodyl tablets which uses a C_{18} chemically bonded silica gel column and ultraviolet (UV) absorption detection at 265 nm. Computerized literature searching located published methods [2–4] for screening of urine by thin-layer chromatography (TLC) to establish laxative abuse in

medical patients, but no papers were found reporting qualitative or quantitative TLC analysis of bisacodyl in pharmaceutical dosage forms. A new quantitative high-performance thin-layer chromatographic (HPTLC) method using automated band-wise sample application, separation on silica gel F plates, and automated scanning of sample and standard zones is described below. Accuracy and precision were excellent, and the new method is faster, more convenient, and uses less solvent than HPLC.

EXPERIMENTAL

Preparation of Bisacodyl Standard Solutions

A 1.00 mg mL^{-1} stock standard solution, used directly for spiking in standard addition accuracy validation analysis, was prepared by dissolving bisacodyl (4,4′-(2-pyridylmethylene)bisphenol diacetate; CAS #603-50-9; Sigma, St Louis, MO, USA; catalog # B-1390) reference standard in absolute ethanol. A 0.100 mg mL^{-1} HPTLC standard solution was prepared by tenfold dilution of the stock solution with ethanol.

Preparation of Sample Solutions

Store brand enteric-coated bisacodyl tablets with a label value of 5 mg were obtained from a pharmacy. Sample test solutions were prepared by grinding a tablet by means of a mortar and pestle, transferring the powder to a 100-mL volumetric flask by use of 70–80 mL ethanol, stirring the solution magnetically for 30 min, sonicating for 20 min, diluting to volume with ethanol, and mixing the contents by shaking. To remove undissolved excipients before application of the sample solutions to the HPTLC plate each solution (approx. 5 mL) was filtered through a Pall Gelman (Ann Arbor, MI, USA) Acrodisc LC 13 mm syringe filter with a 0.45 μm PVDF membrane into a capped vial. The theoretical concentration of the tablet test solutions was 0.0500 μg μL^{-1} bisacodyl.

HPTLC Analysis

Analyses were performed on 20 cm × 10 cm HPTLC silica gel 60 F_{254} plates (EM Science, Gibbstown, NJ, USA, an affiliate of Merck KGaA, Darmstadt, Germany, catalog #13153) with concentrating zone, 19 channels, and fluorescent indicator. Layers were cleaned by predevelopment to the top with dichloromethane–methanol, 1:1, and dried in a fumehood.

Sample and standard zones were applied to the layer as bands by means of a Camag (Wilmington, NC, USA) Linomat IV automated spray-on applicator equipped with a 100-μL syringe and operated with the 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 side edge 6.5 mm, and distance from the bottom of the plate 1.5 cm. For analysis of bisacodyl tablets, duplicate 2.00 μL, duplicate 4.00 μL, and 8.00 μL volumes of the TLC standard solution (0.200–0.800 μg) and duplicate 8.00 μL volumes of the sample solution (containing 0.400 μg bisacodyl according to the label value) were applied to the plate.

Plates were developed to a distance of 6 cm beyond the concentrating zone–silica gel layer interface, with ethyl acetate–methanol–glacial acetic acid, 85:10:5, as mobile phase in a Camag HPTLC twin-trough chamber lined with a saturation pad (Analtech, Newark, DE, USA) and equilibrated with the mobile phase for 15 min before inserting the plate. Approximately 30 mL mobile phase (15 mL in the trough containing the plate and 15 mL in the trough containing the pad) were used for each development, which required approximately 20 min.

After development the plate was dried in air, in a fumehood, for 5 min, and sample and standard zones were quantified by linear scanning at 254 nm by use of a Camag TLC Scanner II with a deuterium source, slit dimension settings of length 4 and width 4, and a scanning rate of 4 mm s^{-1}. The CATS-3 software controlling the densitometer produced a calibration plot by linear regression relating standard zone weights to their scan areas, and the experimental weight of bisacodyl in samples was automatically interpolated from the calibration curve. The recovery (%) was calculated by comparing the mean weight calculated from duplicate sample zones with the theoretical weight according to the label value.

Validation

The accuracy of the new HPTLC method was validated by standard addition analysis. A tablet test solution was prepared by the procedure described above. This solution (1000 μL) was mixed with stock solution (50.0 μL), to double the bisacodyl concentration on the basis of the label value. The original and fortified solutions were analyzed on the same plate by application of duplicate 8.00-μL and 4.00-μL volumes, respectively, and the four standards described above. The difference between the mean experimental weights and the weight added was calculated to determine the accuracy of the method. Precision (repeatability) was validated by

spotting six 8.00-µL volumes of one sample on the same plate and calculating the relative standard deviation (*RSD*) of the experimental weights. As another measure of precision, the difference (%) between the areas of duplicate sample and standard zones applied in each tablet analysis was calculated.

RESULTS AND DISCUSSION

Ethanol readily dissolved bisacodyl standard and completely extracted it from ground tablets treated by the combined stirring and sonication procedure. Insoluble sample excipients did not settle readily to the bottom of the volumetric flask, so the filtration step was adopted to prepare a clear test solution for spotting on the HPTLC plates.

Development of silica gel layers with concentrating zone and containing fluorescent indicator with the mobile phase described above produced compact, flat, fluorescence-quenched bands of bisacodyl (R_F 0.80) against a bright green background when viewed inside a darkroom viewing cabinet under 254-nm UV light. Despite the presence of a variety of inactive ingredients in the tablets (Table I), no other zones were detected in the sample chromatograms. An advantage of using the Linomat and plates with a concentrating zone is that variable volumes of a single standard solution and the sample solutions can be applied, and equal-sized initial zones that lead to accurate and precise densitometric quantification will result (Fig. 1).

Table I

Inactive ingredients contained in bisacodyl tablets (Product 1)

Acacia	Polyvinyl acetate phthalate
Lactose	Silicon dioxide
D&C Yellow No. 10	Sodium starch glycolate
FD&C Yellow No. 6	Stearic acid
Gelatin	Sugar
Magnesium stearate	Titanium dioxide
Microcrystalline cellulose	Wax
Pharmaceutical glaze	

The wavelength of maximum absorption was determined to be 195 nm by measurement of the in-situ UV absorption spectrum of a standard zone of bisacodyl with the Camag scanner in spectral mode. This

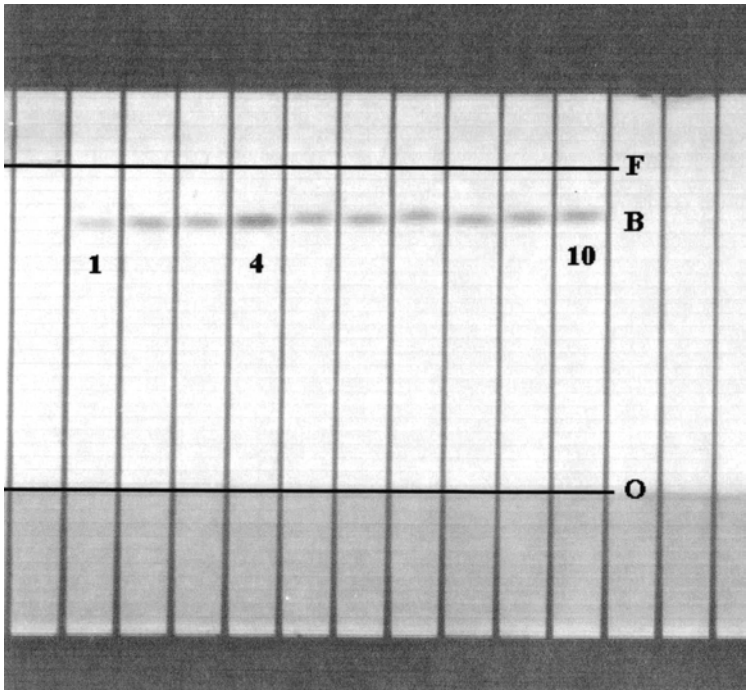

Fig. 1

Chromatograms obtained in precision validation analysis of 5-mg bisacodyl Tablet 7 (Table I, Product 1) on a channeled silica gel plate with concentrating zone by the described HPTLC–densitometry method. The plate was photographed under 254 nm UV light with a Camag VideoStore Image Documentation System. F, mobile phase front; O, origin; lanes 1–4: bisacodyl (B) standards; lanes 5–10, replicate sample volumes. To obtain a clear photograph illustrating the appearance of the zones the applied volumes of standards and samples in the method description were increased by a factor of 5 for this plate. In actual chromatograms used for analyses the zone of the most dilute standard is barely visible to the eye and cannot be seen well in a photograph.

wavelength did not, however, provide quantitative results as accurate and precise as when the secondary absorption maximum of 254 nm was used.

As recommended by the International Committee on Harmonization (ICH) [5], a calibration plot was established by using five analyte concentrations (2.00, 4.00, 6.00, 8.00, and 10.0 μL of the HPTLC standard, containing 0.200–1.00 μg bisacodyl). The linear regression correlation coefficient (*r* value) of this plot was 0.998. For routine analytical procedures, a three point calibration plot within this range was used, produced by applying 2.00, 4.00, and 8.00 μL of the HPTLC standard to each plate. This calibration plot was repeated many times and was also found to have

a linear regression *r* value of 0.998. Although the lowest weight of bisacodyl used for the calibration plot was 0.200 μg, which produced a scan area of approximately 500, the limit of visual detection and quantification were approximately 0.100 μg.

Six bisacodyl tablets were analyzed by the new method with $n = 2$, and one tablet with $n = 6$. The recoveries, compared with the 5 mg label value, are shown in Table II (Product 1). It is apparent that all the samples assayed within the 90–110% specification range declared in the USP for bisacodyl tablets [1].

Table II

Recoveries (%) relative to the 5 mg label value

Sample		Recovery (%)	*n*	RSD (%)
Product 1	Tablet 1	104	2	1.89
	Tablet 2	96.2	2	
	Tablet 3	110	2	
	Tablet 4	109	2	
	Tablet 5	103	2	
	Tablet 6	109	2	
	Tablet 7	99.6	6	
Product 2	Tablet 1	92.8	2	2.88
	Tablet 2	101	2	
	Tablet 3	95.2	2	
	Tablet 4	105	6	

n is the number of sample spots applied to the layer
RSD is the relative standard deviation (coefficient of variation)

Precision was assessed by analyzing one tablet brand six times and calculating the percentage relative standard deviation (coefficient of variation) of the experimental weights. The results, shown in Table II, meet the acceptance criterion specified by the ICH for *RSD* of recovery in assays of finished pharmaceutical products [5]. As another measure of precision the percentage difference between the scan areas of duplicate sample and standard volumes applied in $n = 2$ analyses was calculated. The results ranged from 0.080 to 8.4%, with a mean of 2.6%.

The accuracy of the new method was evaluated by performing a standard addition analysis of an eighth tablet, in which unspiked and spiked solutions were analyzed on the same plate. The unspiked tablet

assayed at 108% of the label value, and analysis of the spiked solution indicated that recovery of the added weight was 100.05%, an error of 0.05%. This result easily meets the acceptance criterion of the ICH [5] for accuracy testing in the assay of finished pharmaceutical products. It was not possible to validate the accuracy of the new method further by determining recovery from a spiked blank solution, because a pharmaceutical tablet containing all or most of the inactive ingredients shown in Table I and an active ingredient that would separate from bisacodyl could not be found to serve as the blank.

It has been shown that the new HPTLC method achieved recoveries as a percentage of tablet label value, standard deviation for replicate analysis, and recovery of the analyte from a spiked standard addition sample that compare favorably with those reported regularly in the literature for HPTLC and HPLC analysis of pharmaceutical dosage forms and required by various regulatory agencies [5]. Sample treatment is simple and there was no interference from excipients. 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 plot, in a single run on the same plate, rather than performing sequential injection of samples and standards in HPLC. Solvent usage on a per-sample basis is also very low. Simultaneous chromatography of samples and standards under identical conditions on the same layer leads to results with excellent accuracy and precision.

The new method will be applicable to other pharmaceutical formulations of bisacodyl containing different inactive ingredients if sample and standard zones and their densitometric scans are as tight and uniform as for the product described above. If the different inactive ingredients cause the analyte zones in sample chromatograms to have irregular shapes, resulting in densitometric scans that do not match the symmetrical scans of the standard zones, an alternative sample preparation procedure and/or mobile phase might be required. For example, a second store brand of 5-mg biascodyl enteric-coated laxative tablets containing the inactive ingredients shown in Table III required extraction with water–acetonitrile, 12.5:87.5 [1] and use of the mobile phase methyl isobutyl ketone–*m*-xylene–hexane–glacial acetic acid, 60:20:12:0.5 [3] (R_F 0.63) for successful analysis using the new method. With only these two changes in the procedure described above, analysis of three tablets with $n = 2$ and one tablet with $n = 6$ gave the recovery and *RSD* values shown in Table II (Product 2). Recovery from a standard addition sample used to verify accuracy was 95.2% compared

with the label value, and recovery of the spike in the re-analysis was 99.33%, an error of 0.67%.

Table III

Inactive ingredients contained in bisacodyl tablets (Product 2)

Calcium sulfate	Methacrylic acid copolymer
Carnauba wax	Poly(ethylene glycol)
Confectioner's sugar	Powdered cellulose
Croscarmellose sodium	Pregelatinized starch
D&C Yellow no. 10	Silicon dioxide
Dibasic calcium phosphate	Sucrose
FD&C Yellow no. 6	Talc
Gelatin	Titanium dioxide
Kaolin	White wax
Magnesium stearate	

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