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Smartphone as a Portable Detector for Thin-Layer Chromatographic Determination of Some Gastrointestinal Tract Drugs

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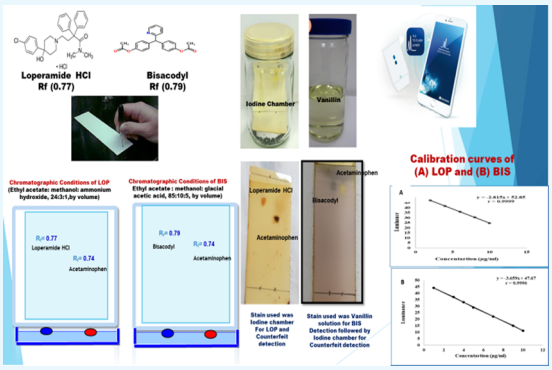
ABSTRACT: Thin-layer chromatography (TLC) is an effective and simple technique for screening, evaluating, and quantifying low-quality and counterfeit pharmaceutical products. Smartphones have recently been used as accessible, cheap, and portable detectors that can replace more complicated analytical detectors. In this work, we have developed a simple and sensitive TLC method utilizing a smartphone charged-coupled device (CCD) camera not only to verify and quantify some gastrointestinal tract drugs, namely, loperamide hydrochloride (LOP) and bisacodyl (BIS), but also to detect acetaminophen (ACT) as a counterfeit drug. Both drugs (LOP and BIS) were chromatographed separately on a silica gel 60 F_{254} plate as a stationary phase under previously reported chromatographic conditions, using ethyl acetate:methanol:ammonium hydroxide (24:3:1, by volume) and ethyl acetate:methanol:glacial acetic acid (85:10:5, by volume) as developing systems to determine LOP and BIS, respectively. Universal stains, namely, iodine vapors and vanillin, were used to visualize the spots on the TLC plates to get a visual image using the smartphone camera and a spotlight as an illumination source with no need for a UV illumination source. The spot intensity was calculated using a commercially available smartphone application for quantitative analysis of the studied drugs utilizing "acetaminophen" as an example of a counterfeit substance. R_f values were calculated using the recorded images and found to be 0.77, 0.79, and 0.74 for LOP, BIS, and ACT, respectively, providing drug identity. Linear calibration curves using the smartphone−TLC method were obtained between the luminance and the corresponding concentrations over the ranges of 2.00−10.00 μg/mL and 1.00−10.00 μg/mL with limits of detection of 0.57 and 0.10 μg/mL for LOP and BIS, respectively. The suggested method was validated according to the International Conference of Harmonization (ICH) guidelines. The method was then successfully applied for the qualitative and quantitative determination of LOP or BIS as an example for gastrointestinal tract drugs in pure form and in their pharmaceutical dosage formulations. The proposed method is considered as a perfect alternative to traditional reported densitometric methods due to its simplicity, easy application, and inexpensiveness. No previously reported methods utilizing smartphones have been published for the determination of the studied drugs. The developed approach is considered the first TLC method using smartphones for the determination of some gastrointestinal tract drugs in their pure form and in pharmaceutical formulations.



1. INTRODUCTION

Recently, widely available detection methods like scanners and smartphones with charged-coupled device (CCD) cameras[1] have been used as a detector and processed the image with commercially available application software.[2] Smartphones compared to the traditional cellphones have more sophisticated interfaces, multidata processing, and higher-resolution lenses. Users at home can easily install applications from application stores, which greatly expands smartphone development. Smartphone-based detection technology has gained popularity due to its portability and low cost compared with commercial instruments and ease of use with no need for instrumentation expert technicians.[2,3] Smartphones enable visualization of TLC plates and thus detect the presence of counterfeit drugs. Iodine vapors and vanillin were used for the TLC plate visualization because they are widely available, economic, and semi-destructive.[4]

Loperamide (LOP) HCl (4-(*p*-chlorophenyl)-4-hydroxy-*N*,*N*-dimethyl-α,α-diphenyl-1-piperidinebutyramide hydro-

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chloride, Figure 1a, LOP) is used to reduce gut motility; thus, it is specified to control symptoms of diarrhea.[5−7] Several

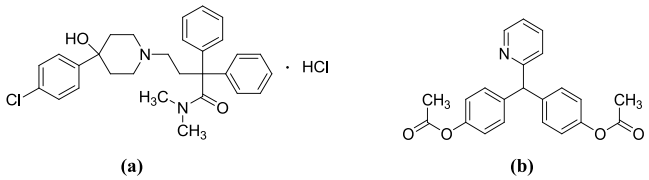

Figure 1. Chemical structure of LOP (a) and chemical structure of BIS (b).

analytical methods were reported to determine LOP including colorimetry,[8] spectrophotometry,[8,9] gas chromatography,[10] conductometry,[11] electrochemical methods,[12,13] high-performance liquid chromatography,[14−20] and TLC-densitometric methods.[21]

On the other hand, bisacodyl (BIS, Figure 1b) is a synthetic pyridinylmethylene-diacetate ester derivative stimulant laxative indicated to treat constipation and bowel irregularity.[22,23] BIS is chemically [4-[(4-acetyloxyphenyl)-pyridin-2-ylmethyl]-phenyl]acetate. Various analytical methods were described to determine BIS, including capillary electrophoresis,[24] liquid and gas chromatography,[25−27] TLC-densitometric methods,[28] spectrophotometry,[29,30] and electrochemical methods.[31,32]

Counterfeit medications containing falsified ingredients, where no active pharmaceutical constituent (APC) is added or the amount of the correct active constituent is substituted by other cheaper and incorrect amounts of APC such as acetaminophen (ACT), are considered one of the most serious risks to patient safety.[33−36] Low amounts or the absence of actual ingredients of GIT drugs substituted or adulterated with low quality ACT may cause gastrointestinal disorders and complications especially in patients who had previous ulcer complications that may result in upper gastrointestinal bleeding/perforation that requires alternative medications.[37,38]

Low-quality pharmaceuticals are considered one of the major difficulties that might have an adverse effect on patient's health as the false drug content might make the patient's condition worse. Therefore, there is a great need to evolve rapid, simple, and low-cost techniques for identifying low-quality pharmaceuticals. Identification of the counterfeit market products can be successfully achieved using TLC, which is a simple and rapid technique to detect and determine quantitatively the active principle ingredients and the false medication.[39−42] TLC detection methods provide both qualitative information (via R_f value determination) and quantitative data via a variety of detection methods.[43] The developed method has advantages compared to some of the reported TLC visualization techniques by using smartphones,[39,44] in that it was the first TLC technique utilizing smartphones to determine some of the gastrointestinal tract drugs (LOP and BIS). Furthermore, counterfeit LOP and BIS in their pharmaceutical dosage forms can be identified by an adulterant (ACT) using two different strains namely, iodine and vanillin.

The aim of the present study was to develop and validate a simple, easy, rapid, and cost-effective TLC method for application in limited resource areas to detect and determine the presence of LOP or BIS as an active ingredient in their pharmaceutical formulations using a smartphone as a detection technique in quality control labs. Furthermore, the study also aimed to detect the falsified or substandard medications adulterated with ACT.

2. EXPERIMENTAL SECTION

2.1. Apparatus. Samsung smartphone note 8 CCD cameras for collection of images, and thin-layer chromatographic plates pre-coated with silica gel 60 (F_{254}, 20 × 20 cm2, 0.25 mm thickness, E. Merck, Darmstadt, Germany) were used.

2.2. Software Used for Detection. Color Picker free software application version 5.0.6 (https://play.google.com/store/apps/details? Id = gmikhail.colorpicker) was used.

2.3. Chemicals and Reagents. *2.3.1. Samples and Reagents.* Loperamide hydrochloride (LOP), bisacodyl (BIS), and acetaminophen (ACT) were obtained from Sigma Aldrich (Cairo, Egypt). Their purities were verified to be 99.24%, 99.50%, and 99.80%, respectively. Iodine, vanillin powder, methanol, ethyl acetate, glacial acetic acid, and 25% concentrated ammonium hydroxide were obtained from Sigma Aldrich (Cairo, Egypt). All chemicals and reagents used were of analytical grade.

2.3.2. Pharmaceutical Formulations. Imodium tablets were purchased from the local market (batch number 8IV133). Each tablet claimed to contain 2.00 mg of loperamide hydrochloride as an active ingredient. The tablets were manufactured by Catalent UK Swindon Zydis Ltd. (Frankland Road, Blagrove Swindon, Wiltshire). Dulcolax tablets were manufactured by Delpharm Reims (France, batch number 190796) and were purchased from the local market. Each tablet claimed to contain 5.00 mg of bisacodyl as an active ingredient.

2.4. Stock and Working Solutions. Accurate weights of LOP, BIS, and ACT equivalent to 10.00 mg were transferred separately into three 10 mL volumetric flasks, and the volume was completed with methanol to obtain final stock solutions (1.00 mg/mL) of the corresponding drugs. Working solutions (2.00−10.00, 1.00−10.00, and 5.00 μg/mL) were freshly prepared by appropriate dilution from the previously prepared stock solutions for LOP, BIS, and ACT with methanol, respectively.

2.5. Iodine Chamber. The iodine chamber was prepared by adding a few crystals of solid iodine with silica powder in a screw-capped TLC chamber.

2.6. Vanillin Solution. Into a 250 mL volumetric flask, about 15.00 g of vanillin powder was transferred and dissolved in a small portion of ethanol and 2.50 mL of concentrated sulfuric acid and then the volume was completed by ethanol. The prepared vanillin stain is light-sensitive and should be stored while wrapped in aluminum foil in the refrigerator.

3. PROCEDURE

3.1. Thin-Layer Chromatographic Conditions. Based on previously reported optimization research, the TLC conditions were optimized for the studied drugs LOP and BIS. Silica gel F_{254} plates act as a stationary phase for both drugs. The mobile phase used was ethyl acetate:methanol:ammonium hydroxide (24:3:1, by volume) for LOP determination,[21] while in the case of BIS, the mobile phase used was ethyl acetate:methanol:glacial acetic acid (85:10:5, by volume).[28]

3.2. Visualization of TLC Plate. First, we immersed the syringe into the LOP or BIS working solutions and ACT, then

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gently touched the end of the TLC plate, and then placed the TLC plate in the previously mentioned chromatographic conditions (Section 3.1). The plate was allowed to develop until the solvent was about 1 cm below the top of the plate and then was gently removed from the beaker and was left to dry.

The TLC plates were then visualized by exposing to iodine vapors and vanillin stain for LOP and BIS, respectively. For LOP, visualization was achieved by placing the developed TLC plate (5×10 cm^2) for 5 min in the iodine chamber till yellow brown spots appeared. Meanwhile, for BIS determination, the developed TLC plate was soaked into the prepared vanillin solution and then the plate was left to dry on a hot plate until violet spots appeared. Within about 7 min, a smartphone camera was used to measure the intensity of each spot color on the TLC plate using the Color Picker free software application version 5.0.6 (https://play.google.com/store/apps/details?Id=gmikhail.colorpicker) and the measured intensity was used for the quantitative analysis and construction of the calibration curves of the studied drugs. It is worth noting that iodine vaporizes easily; therefore, all the images were taken within 5 min once we removed the TLC plates from the developing jar, while in the case of vanillin staining, all the images were collected once the TLC plates were dried on the hot plate as it is more stable than iodine. The rear-facing camera of the smartphone is aligned with a plate guide, which brings the TLC plate into focus and into the camera's field of view. The distance between the camera and the plate is 10 cm.[37] The background of the TLC plate was white, and the image was taken with a spotlight as a source of illumination.

3.3. Construction of the Calibration Curves. Aliquots from LOP and BIS stock solutions (1.00 mg/mL) were transferred accurately into two separate sets of 50 mL volumetric flasks and then completed to volume with methanol to obtain the final concentrations range of 2.00−10.00 μg/mL for LOP and 1.00−10.00 μg/mL for BIS. Construction of the calibration curves was achieved by plotting luminance against drug concentrations, and then the regression equation was computed. The developed smartphone−TLC method was validated according to ICH guidelines.[45]

3.4. Application of the Proposed Smartphone−TLC Method to Determine LOP and BIS in Their Pharmaceutical Formulations. Ten tablets for LOP and BIS were accurately weighed and finely powdered, and the average weight of one tablet was then calculated. One tablet of Imodium and Dulcolax (equivalent to 2.00 and 5.00 mg of LOP and BIS, respectively) were transferred separately into two 100 mL volumetric flasks, dissolved in 60 mL of methanol, and sonicated for 20 min and then the volume was completed to obtain final concentrations of 20.00 μg/mL for LOP and 50.00 μg/mL for BIS. From the previously prepared solutions, 3.00 and 1.00 mL were taken and diluted into two 10 mL volumetric flasks for analyzing LOP and BIS to obtain final concentrations of 6.00 and 5.00 μg/mL for LOP and BIS, respectively.

3.5. Method Validation. Validation of the developed method was achieved according to the ICH guidelines.[45] The evaluated parameters were linearity of the calibration curve, precision, limit of detection (LOD), limit of quantification (LOQ), and accuracy using the smart phone−TLC method.

4. RESULTS AND DISCUSSION

In the present work, a TLC−smartphone based detection method was developed to determine the quality and the quantity of two gastrointestinal tract drugs, namely, LOP and BIS. Smartphones have high-resolution lenses and a wide range of apps that can meet the experimental requirements. As a result, smartphones can be considered a type of portable detection equipment capable of replacing TLC scanners. This technique has many advantages as it is simple, rapid, cost-effective, easily accessible, and appropriate in limited resource areas to check the drug quality with high efficiency and needs neither professional training nor the use of sophisticated instrumentation.[37,46] TLC visualization using a smartphone for the detection of the studied drugs was achieved using conventional TLC plates, which are less expensive than the previously reported HPTLC technique, which is more expensive and not readily available in limited resource areas. We just need an image of a plate representing the chromatographic results with the detected spots for visual comparison of R_f values (identity) and intensities (drug quantity) for qualitative and quantitative determination of the studied drugs compared to using a UV lamp as a TLC visualization method that adds extra cost for detection in limited resource areas. This method of detection is only limited to detecting conjugated and aromatic compounds.[33]

4.1. Visualization of TLC Plates. Universal stains like iodine vapors and vanillin were used for the TLC plate visualization method because they are widely available, economic, and semi-destructive.[4] Iodine strongly reacts with aromatics and unsaturated compounds, while vanillin is used for many aldehydes, ketones, and alcohols.[4]

Linearity was achieved over a concentration range of 2.00−10.00 and 1.00−10.00 μg/mL to determine LOP and BIS, respectively. Under the optimized conditions discussed before, two spots of LOP and ACT appeared; thus, the adulteration of LOP with ACT can be detected rapidly starting from 5.00 μg/mL as presented in Figure 2. LOP appears as a dark brown spot while ACT appears as a light brown spot followed by utilizing a smartphone camera to measure the intensity of each spot color on the TLC plate using the Color Picker free software application version 5.0.6 (https://play.google.com/store/apps/details?Id=gmikhail.colorpicker). Finally, the measured intensity was used to construct the calibration curve of LOP. Meanwhile, in the case of BIS, the same procedure for LOP was carried out but the TLC plate was soaked in the vanillin solution instead and left to dry on a hotplate till BIS appears first as violet spots. Then, the same TLC plate was

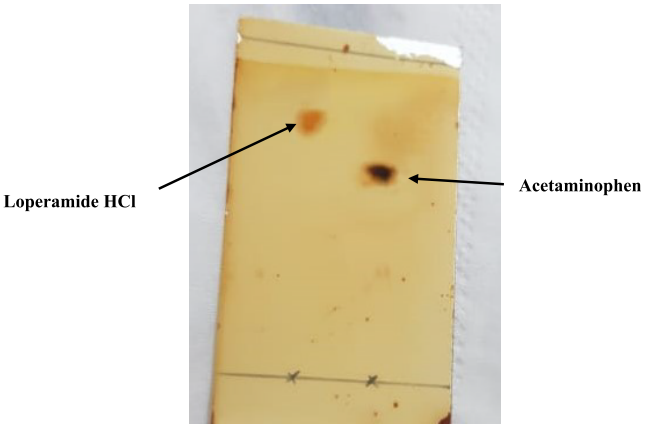

Figure 2. TLC plate of LOP (6.00 μg/mL) using ACT (5.00 μg/mL) as an adulterant visualized using iodine.

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placed in the iodine chamber until ACT finally appeared as a light brown spot as shown in Figure 3. A smartphone camera

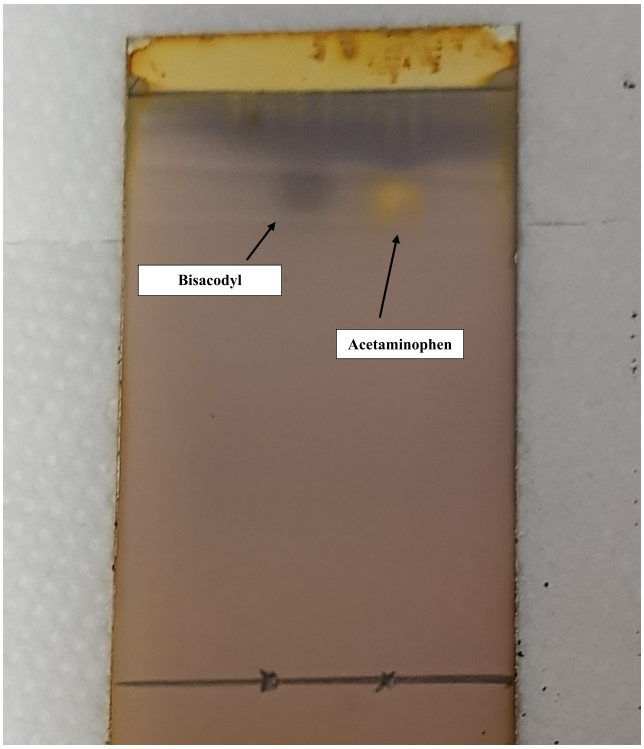

Figure 3. TLC plate of BIS (5.00 μg/mL) using ACT (5.00 μg/mL) as an adulterant visualized with vanillin for BIS detection and then iodine for ACT detection.

was used to measure the intensity of each spot color on the TLC plate using the Color Picker free software application version 5.0.6 (https://play.google.com/store/apps/details?Id=gmikhail.colorpicker), and the measured intensity was used for the quantitative analysis and construction of the calibration curves of the studied drugs then plotting the calibration curve of BIS. The calculated R_f values for LOP, BIS, and ACT are 0.77, 0.79, and 0.74, respectively. Thus, it is very easy and fast to detect ACT as an adulterant (qualitative analysis) just by visualization using vanillin followed by iodine and then determining the R_f of each compound.

4.2. Method Validation. *4.2.1. Linearity, Accuracy, and Precision.* To test the feasibility of the proposed method for the quantitative analysis of the studied drugs, five concentration ranges of LOP were spotted (2.00−10.00 μg/mL) on a TLC plate and the separated spots were visualized using iodine. The luminance of each spot was detected using the software application Color Picker, as presented in Table 1. A linear relationship between calculated luminance and LOP concentration was achieved with r = 0.9999. On the other hand, seven concentration ranges of BIS were spotted (1.00−10.00 μg/mL) on a TLC plate. Then, the separated spots were visualized using vanillin and the luminance of each spot on the recorded image was then detected using the software application Color Picker. A linear relationship between BIS concentration and luminance is achieved with r = 0.9996, as shown in Table 2.

LOD and LOQ were calculated via (SD of response/slope) × 3.3 and (SD of response/slope) × 10, respectively, as presented in Table 2.

Table 1. Relation between Luminance and Different Concentrations of LOP and BIS

drug	conc. (μg/mL)	average luminance[a]	SD	average luminance[b]	SD
LOP	2.00	47.16	0.29	47.20	0.50
	4.00	41.63	0.23	41.33	0.29
	6.00	35.87	0.32	35.70	0.35
	8.00	30.33	0.58	30.50	0.50
	10.00	24.33	0.29	24.33	0.29
BIS	1.00	43.50	0.50	44.03	0.55
	3.00	36.50	0.29	36.87	0.32
	4.00	33.50	0.28	33.17	0.29
	5.00	30.00	0.57	29.50	1.26
	7.00	22.50	0.29	22.50	0.50
	9.00	15.60	0.32	15.37	0.32
	10.00	11.00	0.28	10.83	0.29

[a]Average of 3 replicates on the same spot. [b]Average of 3 replicates on 3 different spots.

Table 2. Validation Parameters of the Developed TLC Visualization Method to Determine LOP and BIS in their Pure Form

parameters	LOP	BIS
concentration range (μg/mL)	2.00−10.00	1.00−10.00
linearity (regression equation)	$Y = -2.815x + 52.85$	$Y = -3.6595x + 47.673$
correlation coefficient[a]	0.9999	0.9996
accuracy (mean ± SD)[b]	100.04 ± 0.48	99.93 ± 1.46
LOD (μg/mL)[c]	0.57	0.10
LOQ (μg/mL)[c]	1.73	0.30
precision (±RSD %)		
Repeatability(μg/mL)[d]	0.76	1.78
Intermediate (μg/mL)[e]	1.10	1.55

[a]Average of three determinations. [b]Average of 5 different concentrations of each LOP and BIS. [c]LOD is calculated via (SD of response/slope) × 3.3 and LOQ is via (SD of response/slope) × 10. [d]Interday precision; the RSD of 3 different concentrations; 4.00, 6.00, and 8.00 μg/mL for LOP and 2.00, 4.00, and 6.00 μg/mL for BIS; 3 replicates each; on the same day. [e]Interday precision; the RSD of 3 different concentrations; 4.00, 6.00, and 8.00 μg/mL for LOP and 2.00, 4.00, and 6.00 μg/mL for BIS; 3 replicates each; on 3 successive days.

4.2.2. Application in Pharmaceutical Formulations. The developed method is sensitive and has been successfully applied to determine LOP and BIS in marketed products. It was observed that the marketed tablet contains pure LOP and BIS where no spot for the adulterant was found indicating 0% ACT. The recovery results were found to be 98.63% and 100.23% for LOP and BIS, respectively, as presented in Table 3.

4.3. Comparison between the Proposed Smartphone−TLC Method with the Reported Methods. Statistical comparison was performed between the results obtained by the proposed smartphone−TLC method and those obtained by applying the reported HPLC method[20] for LOP or the pharmacopeial method for BIS.[47] According to the results obtained, there was no significant difference with respect to accuracy and precision as presented in Table 4.

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Table 3. Determination of LOP and BIS in Imodium and Dulcolax Tablets by Applying the TLC−Smartphone Method

pharmaceutical formulation	recovery% ± SD
Imodium[a]	98.63 ± 1.68
Dulcolax[b]	100.23 ± 1.57

[a]Average of three determinations (claimed to contain 2.00 mg of loperamide hydrochloride). [b]Average of three determinations (claimed to contain 5.00 mg of bisacodyl).

Table 4. Statistical Comparison between the Results Obtained by the Proposed Smartphone−TLC Method and the Reported HPLC Method[20] for the Determination of LOP and Official method for the Determination of BIS[47]

	LOP		BIS	
parameters	developed smartphone−TLC	reported method[20]	developed smartphone−TLC	official method[47]
mean	100.04	99.76	99.93	100.35
SD	0.48	0.42	1.46	2.32
n	5	9	7	6
variance	0.23	0.18	2.13	5.38
F value[a]	1.28	6.04[b]	2.52	4.38[b]
Student's *t* test[a]	1.78	2.26[c]	2.21	2.44[c]

[a]Probability ($P = 0.05$). [b]Tabulated *F* value. [c]Tabulated *t* value.

5. CONCLUSIONS

Development of a simple, rapid, green, low-cost, and portable thin-layer chromatographic plate scanning platform integrated with a smartphone mobile allows direct visualization of the main spots on the plate and thus provides spot intensity and retardation factor that facilitate the quantitative and qualitative detection of the studied drugs, loperamide hydrochloride and bisacodyl. Furthermore, determination of the studied drugs in the presence of possible adulterant acetaminophen was performed. Successful application of the smartphone−TLC method to determine both loperamide hydrochloride and bisacodyl in their pure form and in their pharmaceutical formulations using two different stains, iodine and vanillin. The advantages of the proposed method make it suitable for the analysis of the studied drugs, in quality control laboratories for identifying counterfeit loperamide hydrochloride and bisacodyl in their pharmaceutical dosage forms by its adulterants (ACT) and for easily distinguishing between real and false pharmaceutical dosages. This proposed work provides a new perspective for the quality control of loperamide hydrochloride and bisacodyl.

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Notes

The authors declare no competing financial interest.

This article does not contain any studies with human participants or animals performed by any of the authors.

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