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Combining simplicity with cost-effectiveness: Investigation of potential counterfeit of proton pump inhibitors through simulated formulations using thin-layer chromatography

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ABSTRACT

A simple, accurate and precise high-performance thin-layer chromatographic method has been developed and validated for the analysis of proton pump inhibitors (PPIs) and their co-formulated drugs, available as binary combination. Planar chromatographic separation was achieved using a single mobile phase comprising of toluene: *iso*-propranol: acetone: ammonia 5.0:2.3:2.5:0.2 (*v/v/v/v*) for the analysis of 14 analytes on aluminium-backed layer of silica gel 60 FG_{254}. Densitometric determination of the separated spots was done at 290 nm. The method was validated according to ICH guidelines for linearity, precision and accuracy, sensitivity, specificity and robustness. The method showed good linear response for the selected drugs as indicated by the high values of correlation coefficients (≥0.9993). The limit of detection and limit of quantiation were in the range of 6.9–159.2 ng/band and 20.8–478.1 ng/band respectively for all the analytes. The optimized conditions afforded adequate resolution of each PPI from their co-formulated drugs and provided unambiguous identification of the co-formulated drugs from their homologous retardation factors (hR_f). The only limitation of the method was the inability to separate two PPIs, rabeprazole and lansoprazole from each other. Nevertheless, it is proposed that peak spectra recording and comparison with standard drug spot can be a viable option for assignment of TLC spots. The method performance was assessed by analyzing different laboratory simulated mixtures and some marketed formulations of the selected drugs. The developed method was successfully used to investigate potential counterfeit of PPIs through a series of simulated formulations with good accuracy and precision.

1. Introduction

According to World Health Organization, counterfeit medicines are deliberately and fraudulently mislabelled with respect to identity or source. Their quality is unpredictable as they may contain inadequate amounts of active ingredients, wrong ingredients or have no active ingredients [1]. The wave of counterfeit medicine has hit the whole world as no country either developing or economically developed stands untouched [2]. Their implication can be realized as counterfeit medicines have never missed a spot in the resolution list of World Health Assemblies since 1998. In 2006, WHO launched the International Medical Products Anti-Counterfeiting Task Force (IMPACT) to fight against this issue, which incorporates a voluntary grouping of governments, organizations, institutions, agencies and associations from developing and developed countries [3].

The proton pump inhibitors (PPIs) are acid-activated prodrugs that block the gastric H^+,K^+-ATPase (the proton pump). In presence of gastric acid, they are rapidly metabolized to the active sulfenamide or sulfenic acid by CYP enzymes, mainly CYP2C19 and 3A4, which blocks gastric acid secretion [4]. Omeprazole (OME), lansoprazole (LAN), rabeprazole (RAB) and pantoprazole (PAN) show equivalent efficacy, while esomeprazole (S(−) isomer of OME) and tenatoprazole (TEN) show stronger acid suppression. By inhibition of gastric acid secretion, PPIs helps in curing peptic ulcers, gastroesophageal reflux disease (GERD), Barrett's esophagus, and Zollinger-Ellison syndrome, as well as the eradication of *Helicobacter pylori* as part of combination therapy [4]. Ilaprazole (ILA), a newly approved PPI in Asian countries, is reported to be highly effective and safe for the treatment of Duodenal ulcer [5]. As a matter of fact major PPIs either alone or as combination are available in over-the-counter formulations at relatively low cost. Moreover,

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the existence of several PPIs in the world's top selling drugs has attracted medical counterfeiting thieves [6].

Thin layer chromatography is an economically viable yet a consistent technique for screening of counterfeit drugs. Kovacs et al. [7] have reviewed the performance of three portable planar chromatographic techniques and given them higher suitability score for detecting counterfeit and substandard drugs in resource limited countries. The main objective of this technique was to identify any grossly falsified or substandard drugs. Consequently, they required a minimum of 15–20% bias in order to identify any substandard drug [7]. The proposed techniques including Minilab® made detection of counterfeit medicine simple, convenient, inexpensive yet reliable [8,9]. However none can underestimate the seriousness of the substandard drugs that do not lie within this scope, i.e. the formulations that may not have biased drug content greater than 20% of the claimed value. Minilab® covers analysis of 80 drugs mainly from anti-infective category and a few non-anti-infective ones, but it does not include any of the PPIs and other drugs marketed with PPIs as binary formulations [10]. Moreover, lack of operator's visual sharpness and/or expertise in sample application, Minilab® procedures cannot be used to support a regulatory compliance [11].

As a part of ongoing research on PPIs, we reported a HPTLC method for the separation and estimation of three PPIs from their binary combination with diclofenac in pharmaceutical formulations [12]. In the present work an attempt is made to extend that work to include other co-formulated drugs as well. The chemical structures of all 14 drugs studied are shown in Fig. 1. Literature presents some reports on determination of PPIs alone or in combination formulations by HPTLC [12–14]. As the aims of these methods were to determine two or three drugs simultaneously; they were not able to fulfil the current objective. Moreover, till the date there are no reports on the determination of ILA by planar chromatographic technique. The present study evaluates Herein, we report a unique HPTLC protocol for rapid testing of potential counterfeit of six PPIs along with eight other commercially available co-formulated drugs through a series of laboratory prepared simulated formulations. Table 1 presents a list of all commercially available combinations of PPIs with their co-formulated drugs along with their labelled content. The present method is simple and cost-effective yet sensitive and accurate. The simplicity of the method can be realized as it requires single mobile phase system and detection wavelength for all 14 analytes. The method was also validated as per ICH guidelines [15].

2. Experimental

2.1. Chemicals and materials

Reference standards of diclofenac sodium (DIC, 99.38%) was purchased from Titan Pharmaceuticals Ltd. (Mumbai, India), while omeprazole (OME, 98.71%), pantoprazole sodium sesquihydrate (PAN, 99.55%), rabeprazole sodium (RAB, 98.84%), lansoprazole (LAN, 98.78%), domperidone (DOM, 99.67%) and itopride (ITO, 98.58%) were provided as gift samples by Ashutosh Pellets Ltd. (Gujarat, India). Ilaprazole (ILA, 99.47%), mosapride citrate (MOS, 99.68%), tenatoprazole (TEN, 98.86%), naproxen sodium (NAP, 99.48%), levosulpride (LEV, 98.84%), ondansatron hydrochloride (OND, 98.75%) and alprazolam (ALP, 99.43%) were obtained from Clearsynth Labs (Mumbai, India). Methanol, toluene, acetone, *iso*-propranol and ammonia (25%) used were of analytical grade from E. Merck (Mumbai, India). Pre-coated silica gel 60 GF_{254} HPTLC plates were purchased from E. Merck KGaA (Darmstadt, Germany). After washing with methanol, the plates were pre-activated at 105 °C for 20 min.

2.2. Sample preparation

Separate standard stock solutions for each drug was prepared by dissolving appropriate amounts in a series of 100 mL volumetric flasks (400 μg/mL for ITO, 600 μg/mL for LEV, and 200 μg/mL for all other drugs) in methanol. The stock solutions were suitably diluted with methanol to prepare intermediate and working solutions for each drug. The detailed information regarding solution preparation is given in Supplementary Table 1.

2.3. Chromatography and mass spectrometry conditions

Thin layer chromatography was performed on 10 cm × 5 cm normal phase silica gel 60 GF_{254} HPTLC plates. Sample solutions were applied using a 100 μL Hamilton syringe. Twin-trough chambers (CAMAG, Muttenz, Switzerland) were used for plate development, which was previously saturated with the mobile phase for 15 min. The optimized mobile phase system, consisting of toluene: isopropyl alcohol: acetone: ammonia (5.0:2.3:2.5:0.2, *v/v/v/v*), was used for all the experiments. After successful plate development (up to 7.5 mm distance) under linear ascending method the plates were dried on a plate heater. Linomat 5 auto-sprayer (CAMAG, Muttenz, Switzerland) was used for densitometric scanning mode. Nitrogen aspiration was used at a constant application rate of 15 S/μL under optimized conditions of band length, 6 mm; distance between bands, 14 mm; distance from the plate side edge, 15 mm; and distance from the bottom of the plate, 10 mm. Each of the drugs was visualized as separated spots under UV illumination and was densitometrically analyzed using variable wavelength TLC Scanner 3 (CAMAG, Muttenz, Switzerland). The slit dimensions were length, 5 mm; width, 0.45 mm; and the scanning rate was 20 mm/s. The WinCATS software version 1.4.2 (CAMAG, Muttenz, Switzerland) was used to control the operating parameters, peak area measurements and data processing. Mass spectra were recorder on Waters QDa mass detector (Milford, MA, USA) using electrospray ionization in the positive mode for ALP (*m/z* 309), DOM (*m/z* 426), ILA (*m/z* 367), ITO (*m/z* 359), LAN (*m/z* 370), LEV (*m/z* 342), MOS (*m/z* 422), OME (*m/z* 346), OND (*m/z* 294), PAN (*m/z* 384), RAB (*m/z* 360) and TEN (*m/z* 347) and negative ionization mode for DIC (*m/z* 296) and NAP (*m/z* 229). OriginPro version 8.0724 (OriginLab, MA, USA) was used to prepare some of the graphics.

2.4. Method validation

Validation parameters such as linearity, working range, limit of detection (LOD), limit of quantitation (LOQ), accuracy and precision, robustness were performed as per the ICH guidelines [15].

2.5. Application of the method

Laboratory simulated mixtures of PPIs with all their selected co-formulated drugs together with the excipients were prepared as illustrated in Supplementary Table 2 and then analyzed. A list of common excipients along with their amounts used for all simulated mixtures is given in Supplementary Table 3. Further, a sample containing all the PPIs in a single flask was also prepared to check the applicability of the method to distinguish among selected PPI drugs. From the area response, the performance of the method was assessed in terms of accuracy and precision (% RSD) values.

To test the method for potential counterfeiting, simulated mixtures of PPIs with all their selected co-formulated drugs and excipients were also prepared as illustrated in Table 2 and then analyzed using the developed method. The powdered samples containing the analytes and common excipients were prepared in four different series and coded as S1–7, A1–10, B1–2, C1–3 and D1 (placebo). Series 'S' consisted of standard quantities of drugs as