Regulatory framework on bioequivalence criteria for locally acting gastrointestinal drugs: the case for oral modified release mesalamine formulations

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Abstract

Introduction: Bioequivalence testing for locally acting gastrointestinal drugs is a challenging issue for both regulatory authorities and pharmaceutical industries. The international regulatory framework has been characterized by the lack of specific bioequivalence tests that has generated a negative impact on the market competition and drug use in clinical practice.

Areas covered: This review article provides an overview of the European Union and United States regulatory frameworks on bioequivalence criteria for locally acting gastrointestinal drugs, also discussing the most prominent scientific issues and advances that has been made in this field. A focus on oral modified release mesalamine formulations will be also provided, with practical examples of the regulatory pathways followed by pharmaceutical companies to determine bioequivalence.

Expert commentary: The development of a scientific rationale to demonstrate bioequivalence in this field has been complex and often associated with uncertainties related to scientific and regulatory aspects. Only in recent years, thanks to advanced knowledge in this field, the criteria for bioequivalence assessment are undergoing substantial changes. This new scenario will likely result in a significant impact on pharmaceutical companies, promoting more competition through a clearer regulatory approach, conceived for streamlining the demonstration of therapeutic equivalence for locally acting gastrointestinal drugs.

1. Introduction

A generic medicine is a pharmaceutical product, which has the same qualitative and quantitative composition in active compounds and the same pharmaceutical form as the reference medicinal product, while the inactive ingredients can vary [1,2]. A generic product must be both pharmaceutically equivalent and bioequivalent to the reference medicinal product to allow bridging of preclinical testing with clinical trials that granted marketing authorization of the reference product [3,4]. Both in the European Union (EU) and United States (US), bioequivalence (BE) studies play a crucial role in the regulatory approval of systemically absorbed generic medicines. BE is defined as the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action, when administered at the same molar dose under similar conditions in an appropriately designed study [5].

On this basis, BE evaluations can be employed at regulatory level as surrogate of comparative clinical trials to demonstrate that two medicinal products are therapeutically equivalent in terms of safety and efficacy profiles [5,6].

International regulatory agencies have issued recommendations to define the accepted methodologies for assessing compliance to BE criteria during the development of a generic drug. Different methodological approaches, suitable for the assessment of therapeutic equivalence, have been recommended by the US FDA: comparative clinical trials, pharmacokinetic (PK) BE, pharmacodynamic BE, and in vitro studies [7]. In Europe, the accepted methods for assessing therapeutic equivalence are the same as the US ones; however, only medicinal products that demonstrate BE by PK studies can be considered as generic products, while in other cases, the medicinal product can be authorized through hybrid applications [4].

In general, for systemically absorbed drugs, the scientific and regulatory framework is well established and PK studies still represent the gold standard approach to demonstrate BE for generic drugs [5,6]. However, when considering locally acting drugs, with particular regard for oral gastrointestinal (GI) products, which are characterized by peculiar biopharmaceutical properties, measuring concentrations of the active compound at the site of action through conventional BE methods is more difficult, or practically impossible [8-10]. Therefore, pharmaceutical companies, who intend to develop a generic product, must identify the most appropriate...
equivalence methods to ensure a sensitive and accurate detection of putative in-vivo differences between the test and originator product. In this context, the development of a regulatory strategy has been more complex than for systemic absorbed drugs, and it has been often associated with uncertainties related to scientific and regulatory aspects. These issues have strongly affected the availability of GI generic products in the pharmaceutical market, thus reducing competition and economic savings for both patients and National Health Services [11].

Based on the above background, the aim of this review article is to appraise the state of the art of the regulatory bases underlying the assessment of BE for medicinal products acting locally in the GI tract, highlighting the advances made in EU and US. However, as drugs belonging to this class are characterized by peculiar pharmaceutical and clinical features, we will focus on products that have been frequently associated with challenges related to BE testing, such as oral modified release mesalamine (5-aminosalicylic acid [5-ASA]) and steroids formulations. Indeed, 5-ASA displays a complex biopharmaceutical profile that reflects the majority of issues associated with the demonstration of BE for locally acting GI products. The suitability of clinical efficacy or PK studies for assessing BE of oral 5-ASA formulations has been matter of debate, with much discussion revolving around their potential correlation with local concentrations achieved by 5-ASA at the site of action, or even its release behavior from oral formulations throughout the GI tract [8–12]. Only in recent years, thanks to the scientific and regulatory advances made in this field, the criteria for BE assessment of locally acting GI products are undergoing substantial changes, mainly in EU. Novel BE criteria have been recommended by regulatory agencies and several research activities have been promoted to develop alternative methods, suitable for detecting putative differences in the in-vivo behavior of locally acting GI drugs. The BE issues that will be discussed for oral modified release 5-ASA formulations are also valid for locally active oral steroids, such as budesonide that is used for the treatment of inflammatory bowel disease [13]. Budesonide is a glucocorticoid with topical anti-inflammatory action that has a high first-pass elimination resulting in a low systemic bioavailability and less systemic activity than conventional corticosteroids. One of the main aspects that support the rationale behind the clinical use of budesonide in inflammatory bowel disease is based on the local delivery of the active compound in the inflamed GI mucosa, preventing as much as possible the systemic absorption of the drug [14,15]. Similarly to 5-ASA, various modified release formulations have been developed with the last innovation represented by the Multi Matrix System technology that allows the release of the drug at a controlled rate throughout the colon. As will be reported for 5-ASA, the PK profile of budesonide is complex. After administration, a fraction of the active ingredient exerts a local action in the GI tract and a fraction becomes detectable in the systemic circulation leading to uncertainties during the development of a strategy for BE purpose [14,15]. For these reasons, the regulatory framework and the recommendations provided for oral 5-ASA dosage forms may also be applied for the development of generic version of modified release budesonide formulations [13].

2. BE of locally acting GI drugs

The demonstration of BE for locally acting GI drugs has generated hard challenges since many years. In this setting, the definition of standardized BE criteria has been more difficult than for systemically absorbed products. In general, locally acting GI drugs are conceived for delivering the active ingredient within the gut lumen and are formulated to prevent as much as possible its systemic absorption. Consequently, for these products, the performance of their pharmaceutical forms is crucial, since dissolution is the primary determinant of the rate and extent to which the active ingredient is delivered to the site of action and exerts its therapeutic effect [9]. In this context, the assay of drug concentration in the systemic circulation might not be relevant for BE purposes. Even if a locally acting GI drug generates a measurable systemic PK profile, it is necessary to evaluate if and how these plasma concentrations correlate with the availability of the active ingredient at the site of action. These aspects are crucial to define whether systemic PK end points are relevant for BE purpose, and whether the scientific rationale of BE applied for systemically absorbed drugs can be considered as valid also for locally acting drugs [8,16–18]. In order to better address the above issues, we provide here some examples of locally acting GI drugs widely employed in clinical practice, with the purpose of discussing how their biopharmaceutical and PK features can impact on the process of BE demonstration. In general, three different scenarios for locally acting GI drugs can be hypothesized: (1) no systemic exposure; (2) minimal systemic exposure; (3) measurable PK profiles, but the gut region of absorption may or not overlap with the site of action in the human GI tract [19]. Among locally acting GI drugs, each medicinal product differs from others in terms of mechanism of action, system of drug delivery, and physico-chemical properties (Table 1) [8]. Therefore, a BE method should be selected in accordance with the specific features of the test medicinal product, and the application of general BE criteria, as for systemically absorbed products, may not be recommendable [8]. Over the past, clinical studies have been requested to compare different drug products for purposes of BE demonstration. However, increasing advances in the knowledge of pharmaceutical and pharmacological features of locally acting drugs have led to improvements in the processes of BE evaluation, thereby fostering the development of alternative BE methods, able to document with high accuracy similarity profiles and in-vivo differences between different medicinal products.

Medicinal products that generate minimal or not measurable systemic concentrations, such as Lanthanum carbonate, require a BE approach based on other methods than PK studies. In this case, the FDA recommended that in vitro dissolution tests and phosphate binding studies or pharmacodynamic end points in human subjects should be employed when developing a generic product [29,30]. Indeed, the implementation of comparative dissolution studies allows to reveal potential differences in the manufacturing process that can affect the deliver and availability of the active ingredient at the site of action. Conversely, phosphate binding studies are carried out to assess comparatively whether the generic and
Table 1. Examples of locally acting gastrointestinal medicinal products.

<table>
<thead>
<tr>
<th>Drug and dosage form</th>
<th>Product category</th>
<th>Therapeutic indication</th>
<th>Rate of systemic absorption</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colesevelam Tablet or oral suspension</td>
<td>Binding agents</td>
<td>Reduces elevated low-density lipoprotein cholesterol in adults with primary hyperlipidemia as well as boys and postmenarchal girls with heterozygous familial hypercholesterolemia</td>
<td>Minimal or trace tissue concentrations</td>
<td>[8,20]</td>
</tr>
<tr>
<td>Lanthanum carbonate Chewable tablet</td>
<td>Binding agents</td>
<td>Treatment of hyperphosphatemia in patients with end stage renal disease</td>
<td>0.002%</td>
<td>[8,21,22]</td>
</tr>
<tr>
<td>Vancomycin Capsule</td>
<td>High soluble immediate release dosage forms</td>
<td>C. difficile-associated diarrhea</td>
<td>No measurable concentrations in blood</td>
<td>[8]</td>
</tr>
<tr>
<td>Orlistat Capsule</td>
<td>Low soluble immediate release dosage forms</td>
<td>Enterocolitis caused by Staphylococcus aureus</td>
<td>&lt;2%</td>
<td>[8,23,24]</td>
</tr>
<tr>
<td>Mesalamine Capsule and tablet</td>
<td>Modified release products</td>
<td>Treatment of ulcerative colitis</td>
<td>20–30%</td>
<td>[8,25]</td>
</tr>
<tr>
<td>Budesonide Capsule and tablet</td>
<td>Modified release products</td>
<td>Treatment of ulcerative colitis and Crohn’s disease</td>
<td>10–20%</td>
<td>[26–28]</td>
</tr>
</tbody>
</table>

When considering the case of more complex drugs, such as 5-ASA or budesonide, for which a fraction of the active ingredient exerts a local action in the GI tract and a fraction becomes detectable in the systemic circulation, selecting the most accurate BE method is more challenging. Clinical trials based on efficacy endpoints have been employed in the past to compare oral 5-ASA products, even though they are generally characterized by low sensitivity and are rather expensive [11]. Conversely, clinical PK studies have been used for safety evaluations, but whether the estimated 5-ASA plasma concentrations correlate with its bioavailability at the local site of action, and whether systemic PK data could be taken as surrogate end points for the assessment of BE, remains a matter of debate [8]. Several additional factors, such as intrasubject variability of 5-ASA plasma concentrations and the influence of physiological GI parameters, amplify further the degree of complexity and increase the risk of failing BE demonstration [31]. Moreover, the paucity of studies comparing the local GI concentrations of 5-ASA released by different oral formulations adds more uncertainty on their potential differences in clinical efficacy and interchangeability [31,32]. All the above issues have been discussed for long time both in the scientific literature and at regulatory level, highlighting the need for generating further evidence about the BE testing of oral 5-ASA formulations [12,31,32].

3. 5-ASA case study

5-ASA is a derivative of salicylic acid employed since many years for the therapeutic management of inflammatory bowel diseases, with particular regard for mild-to-moderate ulcerative colitis [33]. After oral or rectal administration, 5-ASA acts locally in the colon and its effectiveness is related with concentrations achieved in the bowel mucosa [33]. The exact mechanism of action of 5-ASA is currently unclear [8]. However, several putative therapeutic targets have been proposed. These include, among the others, the peroxisome proliferator-activated receptor gamma [34,35], the inhibition of cyclo-oxygenase and lipooxygenase with consequent blockade of prostanoid end leukotriene production [35–37]. Recent data suggest also that 5-ASA can interfere with other inflammatory pathways, such as nuclear factor-kappa B activation, thus inhibiting the release of tumor necrosis factor and interleukin-1 [38–41]. Other possible mechanisms comprise the alteration of GI bacterial flora and antioxidant/scavenger activity [42,43]. As 5-ASA is believed to exert a direct effect on colonic mucosa through a variety of anti-inflammatory mechanisms, direct application of this agent to the mucosa of this gut region is required [44,45]. Free 5-ASA, if administered orally, is completely adsorbed from the proximal small intestine, followed by extensive metabolism to N-acetyl-5ASA by the N-acetyltransferase 1 in intestinal epithelial cells and the liver, and then excreted in the urine as a mixture of free 5-ASA and N-acetyl-5ASA [46].

Therefore, to carry out its topical activity, 5-ASA must be delivered to ensure its direct contact with the mucosal layer affected by the inflammatory process, in the distal ileum and colon, while avoiding its early systemic absorption in the upper GI tract. This goal has been pursued with the development of different types of drug delivery technologies: delayed-release products, extended release products, prodrugs, and topical products [47]. Oral modified release products are the most widely prescribed dosage forms of 5-ASA and have captured the main market share [48,49]. The implementation of these technologies allows the delivery of 5-ASA in close proximity of the site where its pharmacological action is required, with a consequent low systemic bioavailability, about 20–30% of 5-ASA detectable in plasma [8,25], and a better safety and efficacy profile. The last innovation was introduced some years ago with the development of Multi Matrix System technology. The latter product is a high-strength formulation of 5-ASA (1.2 g of 5-ASA per tablet), designed for once-daily administration to enhance patient’s compliance and simplify the treatment schedule [50]. This technology allows to release 5-ASA to the full length of the colon by delayed and controlled release mechanisms. This goal is achieved through a delivery system based on gastroresistant
coating and a tablet core containing hydrophilic and lipophilic excipients. When the outer layer of the tablet begins to dissolve at pH 7 or higher, the lipophilic excipients reduce the aqueous penetration and the dissolution of the active ingredient. The core matrix, in contact with intestinal fluids, causes the formation of a viscous gel mass, thus providing a controlled release of 5-ASA throughout the colon [51].

All these 5-ASA products have been formulated to target the inflamed bowel area, beyond the proximal intestine. Accordingly, the release profile of 5-ASA from enteric-coated tablets is the dominant factor for achieving an adequate local bioavailability [44,46] that is strictly correlated with the clinical efficacy [25,52–54]. Clinical evidence demonstrated that the concentration of 5-ASA at the site of action is inversely correlated with the severity of endoscopic and histological activity scores in patients with ulcerative colitis. Patients with higher mucosal concentration of 5-ASA had lower or none colonic inflammation than those with severe activity of ulcerative colitis [52–54]. While these products have displayed good safety and efficacy profiles, differences among the technologies employed for the development of pharmaceutical forms, in conjunction with intra-subject variability, could lead to differences in drug-release profiles, systemic PK profiles, and drug concentration at the site of action [8,44,55–57]. Taken together, all these sources of variability increase further the complexity of assessing BE for modified release 5-ASA products.

### 3.1. Available BE methods for oral modified release 5-ASA products

Local concentrations of 5-ASA at the site of action are crucial for ensuring its therapeutic action in the inflamed bowel area. Therefore, several methods have been developed to characterize the biopharmaceutical properties of 5-ASA products. Since several techniques have been discussed extensively in literature, only their most relevant features will be summarized in this section [12,25]. An ideal method should allow a careful evaluation of the behavior of oral modified release 5-ASA formulations and the delivery of the active ingredient into the colon to unravel putative differences between different 5-ASA dosage forms.

#### 3.1.1. In vitro dissolution tests

The dissolution test is a key tool routinely employed by the pharmaceutical industry for evaluating the performance characteristics of solid oral dosage forms. It is successfully used both for quality control purpose and as relevant part of the BE studies performed for the development of generic medicine.

The dissolution test assesses the amount of drug dissolved in a known volume of liquid medium at a predetermined time, using a specified apparatus designed to control the parameters of dissolution testing [6]. It provides useful data for the optimization of drug delivery and formulation design during the early phase of drug development or for supporting changes made to the manufacturing process [6]. It is also used as a surrogate test to predict the in-vivo performance of drug products, when applicable, and to get information on the test batches used in BE and pivotal clinical studies. From a regulatory point of view, it plays a major role in generic medicine development since it is used to waive BE studies or to assess pharmaceutical equivalence between test and reference product [6]. Therefore, in vitro dissolution studies represent the primary scientific and regulatory step that allows to obtain relevant information about the potential GI behavior of a drug product. The experimental condition is a key factor to simulate the physiological conditions of the GI tract and obtain a good chance of predicting the in-vivo performance of pharmaceutical forms. During the early stage of generic drug development process, comparative dissolution tests are crucial for an early detection of potential differences in the in-vivo GI behavior among different 5-ASA products [8,12,25]. However, it is generally acknowledged that compendial dissolution methods cannot adequately predict the in-vivo release behavior of locally acting GI drugs, since they cannot mimic all variables affecting GI functions, such as the physicochemical properties, composition and volume of luminal fluids in different gut regions, the residence time of the dosage unit in each GI segment, the mechanical forces resulting from GI motility, and many other factors [8,16,25,56,57].

#### 3.1.2. PK studies

The systemic PK profile of modified release 5-ASA products is well known and has been extensively discussed in previous articles [25,46]. Briefly, it is acknowledged that about 20–30% of the total 5-ASA dose administrated trough oral modified release formulations is absorbed and can be detected in plasma [8,25,46]. Therefore, PK parameters are helpful for the evaluation of the safety profile of 5-ASA products. Conversely, the role of systemic bioavailability as surrogate end point for demonstrating BE of different 5-ASA products has been matter of much debate. However, in general, traditional PK BE studies are not sufficient to support the demonstration of similarity of these products, since 5-ASA plasma concentrations reflect the absorption throughout the GI system, and not specifically at the site of action [8,11,16,25]. Nevertheless, as discussed in the following sections, the implementation of partial AUC in the experimental design of BE studies is useful to correlate 5-ASA plasma profiles with its concentration at the site of action in the colon and to define the therapeutic equivalence between generic and reference products.

#### 3.1.3. Clinical studies of therapeutic equivalence

For many years, comparative trials based on clinical endpoints have represented the main methodological approach to the determination of BE among 5-ASA products. However, more recently, there has been a paradigm shift concerning the scientific and regulatory value of these studies in the assessment of BE. Indeed, the majority of regulatory agencies have judged clinical trials as the least sensitive method to detect differences in the pharmaceutical performance of locally acting drugs, such as 5-ASA [8,11]. These studies are also very expensive and require large numbers of patients to obtain clinically and statistically significant results. Based on these drawbacks, alternative BE methods that could be able to unravel in-vivo GI differences among 5-ASA formulations have been developed and validated [8,11].
3.1.4. Pharmacoscintigraphic studies

Scintigraphic techniques can provide useful information on the in-vivo performance of oral formulations, such as the transit rate and the site where the erosion of the dosage forms and the release of the active ingredient start, without the limitations that affect in vitro dissolution tests. This methodology has been employed, alone or in combination with conventional PK studies, as a part of clinical data produced for the authorization of new medicinal products as well as to compare the in-vivo release profiles of different 5-ASA formulations [58–63]. However, even if scintigraphy allows a direct visualization of drug release within the gut lumen, the images may not be useful to estimate the amount of released 5-ASA or to document whether the release of the active ingredient occurs at different rates than that of radiolabeling. Therefore, scintigraphy cannot provide accurate data on the distribution of 5-ASA after its release from the formulation. Accordingly, scintigraphy alone is not regarded as a suitable tool to document all the aspects relevant to BE determination [25].

4. The regulatory framework

BE testing for locally acting GI medicines is a challenging issue for both pharmaceutical industries and global regulatory authorities. In general, the regulatory approval process for generic locally acting GI products has been more complicated than systematically acting ones. The FDA and EMA have released guidelines dedicated specifically to locally acting drugs in an attempt of providing recommendations for pharmaceutical companies. However, the activities implemented by FDA and EMA in this context differ in several respects. Improvements have been introduced, both from a scientific and regulatory standpoint, by the FDA, with the development of specific guidelines and the promotion of new research activities. By contrast, for long time, only general recommendations have been issued by the EMA and other European National Agencies. Only in April 2017, the EMA has published a draft guideline that introduces the possibility of alternative approaches to traditional comparative clinical studies [64]. This regulatory scenario has lead to quite different therapeutic equivalence and authorization pathways for oral modified release 5-ASA formulations in EU and US, as shown in Table 2. Only few relevant regulatory cases are available for the purposes of this review. In US, oral modified release 5-ASA products are still covered by patents [67]. In EU, only one assessment report for an oral modified 5-ASA product, relevant for the scope of this review, is available [68].

Table 2. Bioequivalence criteria adopted in US and EU for the development and authorization of 5-ASA delayed release formulations.

<table>
<thead>
<tr>
<th>Drug product</th>
<th>Bioequivalence approach adopted</th>
<th>Year of approval</th>
<th>Approving countries</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-ASA delayed release capsules</td>
<td>In vitro comparative dissolution test</td>
<td>2013</td>
<td>United States</td>
<td>[65]</td>
</tr>
<tr>
<td></td>
<td>PK study based on the evaluation of conventional PK parameters (C\textsubscript{max} and AUC) in addition to partial AUC\textsubscript{4–48}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-ASA delayed release tablets</td>
<td>First step: In vitro comparative dissolution test and PK studies</td>
<td>2010</td>
<td>United Kingdom and Germany</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Second step: combined pharmacoscintigraphic and PK analysis, single-dose PK study, and phase III comparative clinical efficacy study</td>
<td></td>
<td></td>
<td>[66]</td>
</tr>
</tbody>
</table>

5-ASA: 5-aminosalicylic acid; PK: pharmacokinetic; AUC: area under the curve; C\textsubscript{max}: maximum concentration.

4.1. FDA regulatory view

The US regulation 21 CFR 320.24, addressing the methodologies eligible for BE evaluation, includes in-vivo PK studies, in-vivo pharmacodynamic studies, clinical endpoint studies, in vitro studies, and any other approach deemed adequate [7]. The general background recommendation is to employ the most accurate, sensitive, and reproducible method according to the specific features of the drug product. However, for locally acting GI drugs, clinical studies have been recommended for BE demonstration, even if the FDA regards this approach as ‘the least accurate, sensitive, and reproducible of the general approaches for measuring bioavailability or demonstrating bioequivalence’ [7,8]. Over the years, this regulatory paradigm has been largely modified, and considerable advances have been made in the research activities promoted by the FDA for the development of alternative BE approaches [10]. In 2010, the FDA has issued product-specific recommendations for BE studies [69]. The definition of product-specific recommendation has delineated a clear regulatory framework that helps pharmaceutical companies in the development of scientific evidence in line with the expectations of regulatory agencies [70]. To date, 1554 guidances, covering any type of medicinal products, have been released. In this context, several recommendations for locally acting GI products have been issued [70]. These guidances (although not mandatory) represent an important aid to pharmaceutical companies for the development of generic drugs, with particular regard for those exerting a topical action. It is evident how the FDA approach is structured and oriented to provide specific recommendations that reflect actually the features of the medicines under investigation, thus allowing to simplify and shorten the development of generic medicines.

4.1.1. BE recommendations for oral modified 5-ASA products in US

The paradigm change implemented by the FDA about the BE assessment for oral modified release 5-ASA products could be related to two different citizen petitions submitted to the US agency [71]. One of the main issue raised by these petitions focused on the role of PK studies for BE assessment of the generic versions of modified release 5-ASA formulations. The FDA stated that standard PK parameters, such as AUC and C\textsubscript{max} (maximum concentration), are not sufficient to discriminate between two 5-ASA products. Conversely, however, the agency recommended the use of partial AUC in addition to conventional parameters, arguing that, if plasma 5-ASA concentrations are monitored over specified time intervals, it is
possible to evaluate the absorption rate at the site of action. Accordingly, the FDA assumes that the combination of comparative in vitro dissolution tests and properly designed PK studies, under fast and fed condition, are suitable for detecting any difference in the drug release, and estimating if two 5-ASA products are absorbed at the same rate and extent at the site of action [71]. Overall, both citizen petitions have lead to the release of specific recommendations for the BE demonstration of modified release 5-ASA products. Table 3 provides a summary of BE criteria recommended by the FDA in the draft guidance for oral modified release 5-ASA products. Further information about the design of PK studies or the experimental conditions required for other kinds of locally acting GI drugs, such as rectal 5-ASA products, cholestyramine, lanthanum carbonate, and acarbose, are available in the FDA website section dedicated to Product-Specific Recommendations for Generic Drug Development [70].

### 4.2. EMA regulatory view

The first guidance released by the EMA in 1995 was the ‘Note for guidance on the clinical requirements for locally applied, locally acting products containing known constituents’ [78]. This guideline covers all type of locally acting medicinal products without making specific reference to locally acting GI drugs. In principle, the regulatory strategy suggested by the EMA for the demonstration of equivalence for ‘generic’ locally acting products relies on the conduction of comparative efficacy clinical trials. However, depending on the type of medicinal product, the EMA allows other BE approaches, provided they are validated adequately. Indeed, the application for the approval of a generic product should be supported by a documentation consisting of pharmacodynamic studies or local bioavailability studies; possibly in vitro studies or augmentation in case of minor differences. Otherwise clinical studies. Any safety issue has to be addressed appropriately. The choice of the most appropriate method and the omission of such data should be justified by the pharmaceutical company. [78]

If we consider the heterogeneity of medicinal products belonging to the GI class, these recommendations sound as very general and may lead to different interpretations, depending on the type of product. These difficulties have been pointed out by the EMA in 2013 in the ‘Concept paper on the development of a guideline on the demonstration of therapeutic equivalence for locally applied and locally acting products in the gastrointestinal tract’ [79]. In this paper, the EMA acknowledged that clinical or pharmacodynamics end points may not be able to predict and detect differences between locally acting products. At the same time, they agreed that the in vitro pharmaceutical properties of these medicinal products are relevant for the purpose of BE demonstration. On these bases, the Agency underscored the need for establishing alternative in vivo and in vitro methods for BE evaluation [79]. It is surprising that this scenario was the main regulatory reference for over 20 years. Unfortunately, the lack of univoque interpretation of this guideline has had a negative impact on the regulatory strategy for generic/hybrid drug development. A similar trend has occurred for the definition of product specific recommendations for BE purposes. In 2013, the EMA released the ‘Concept paper on the development of product-specific guidance on demonstration of bioequivalence’, which was then converted into an approved guidance [80]. In this guidance, the first approach focused specifically on oral immediate release formulations. To date, 42 guidelines have been released. However, no product-specific guidance for locally acting GI drugs has been issued by the EMA [81].

#### 4.2.1. The new EMA draft guidance on the demonstration of therapeutic equivalence for locally acting GI drugs

In April 2017, a ‘Draft guideline on equivalence studies for the demonstration of therapeutic equivalence for products that are locally applied, locally acting in the gastrointestinal tract as addendum to the guideline on the clinical requirements for locally applied, locally acting products containing known constituents’ has been issued by the EMA [64]. This draft guidance updates the guideline published in 1995. At present, it is open for consultation, and the dead line for this phase falls on 30 September 2017. This new draft guideline reflects the beginning of a substantial change in the regulatory paradigm driving this area, introducing the possibility of demonstrating therapeutic equivalence of locally acting GI drugs through

### Table 3. Bioequivalence criteria for oral modified 5-ASA formulations recommended by the FDA.

<table>
<thead>
<tr>
<th>Pharmaceutical form and strength</th>
<th>In vitro dissolution studies</th>
<th>PK endpoints</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delayed release tablet 400 mg</td>
<td>Stage 1: acid pH</td>
<td>AUC&lt;sub&gt;Fd&lt;/sub&gt;, AUC&lt;sub&gt;Fd&lt;/sub&gt;-max</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td>Stage 2: 6 different pH values from 4.5 to 7.5</td>
<td>Other partial AUCs may be evaluated</td>
<td></td>
</tr>
<tr>
<td>Delayed release tablet 800 mg</td>
<td>Stage 1: acid pH</td>
<td>AUC&lt;sub&gt;Fd&lt;/sub&gt;, AUC&lt;sub&gt;Fd&lt;/sub&gt;-max</td>
<td>[73]</td>
</tr>
<tr>
<td></td>
<td>Stage 2: 6 different pH values from 4.5 to 7.5</td>
<td>Other partial AUCs may be evaluated</td>
<td></td>
</tr>
<tr>
<td>Delayed release tablet 1200 mg</td>
<td>Pretreatment stage 1: acid pH</td>
<td>AUC&lt;sub&gt;Fd&lt;/sub&gt;, AUC&lt;sub&gt;Fd&lt;/sub&gt;-max</td>
<td>[74]</td>
</tr>
<tr>
<td></td>
<td>Pretreatment stage 2: basic pH</td>
<td>Other partial AUCs may be evaluated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Evaluation stage: 4 different pH values from 6.5 to 7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed release capsule 400 mg</td>
<td>Stage 1: acid pH</td>
<td>AUC&lt;sub&gt;Fd&lt;/sub&gt;, AUC&lt;sub&gt;Fd&lt;/sub&gt;-max</td>
<td>[75]</td>
</tr>
<tr>
<td></td>
<td>Stage 2: 6 different pH values from 4.5 to 7.5</td>
<td>Other partial AUCs may be evaluated</td>
<td></td>
</tr>
<tr>
<td>Extended release capsule 375 mg</td>
<td>Stage 1: acid pH</td>
<td>Fasting: AUC&lt;sub&gt;Fp&lt;/sub&gt;, AUC&lt;sub&gt;Fp&lt;/sub&gt;-max</td>
<td>[76]</td>
</tr>
<tr>
<td></td>
<td>Stage 2: 6 different pH values from 4.5 to 7.5</td>
<td>Fed: AUC&lt;sub&gt;Fp&lt;/sub&gt;, AUC&lt;sub&gt;Fp&lt;/sub&gt;-max</td>
<td></td>
</tr>
<tr>
<td>Extended release capsule 500 mg</td>
<td>Stage 1: acid pH</td>
<td>Fasting: AUC&lt;sub&gt;Fp&lt;/sub&gt;, AUC&lt;sub&gt;Fp&lt;/sub&gt;-max</td>
<td>[77]</td>
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<tr>
<td></td>
<td>Stage 2: 6 different pH values from 4.5 to 7.5</td>
<td>Fed: AUC&lt;sub&gt;Fp&lt;/sub&gt;, AUC&lt;sub&gt;Fp&lt;/sub&gt;-max</td>
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AUC: area under the curve; C<sub>max</sub>: maximum concentration.
The BE package comprises three studies: in vitro comparative dissolution test and in-vivo fast and fed PK studies for each type of formulation. A reference-scaled average BE approach is recommended for each type of dosage form.
alternative models than comparative clinical or PD end points. A more specific scientific and regulatory approach is proposed, with several additional clear recommendations, based on the characteristics of drug products, such as the site and mechanism of action, their biopharmaceutical and PK properties, the pharmaceutical form, and the state of the active ingredient within the dosage form. For each class of GI drugs, the EMA proposes a decision tree that indicates clear and useful regulatory and experimental strategies that should be taken properly into account. The approach proposed by the EMA allows to follow several regulatory pathways requiring different levels of scientific evidence to demonstrate therapeutic equivalence. These strategies include 'pharmaceutical quality data alone; pharmaceutical quality data + in vitro model; pharmaceutical quality data plus in vivo PK data; pharmaceutical quality data plus in vitro model + in vivo PK data'. The selected strategy should be adequately justified according to the drug product characteristics.

The EMA recognizes that, in some instances, in vitro and in-vivo methods may display better sensitivity than clinical or PD end points to reveal potential differences between medicinal products containing the same active substance. It is acknowledged that these methods could be employed to waive clinical trials, provided the applicant justifies adequately that they are able to reflect the in-vivo drug release and availability at the site of action. The regulatory strategies and methods accepted for demonstration of therapeutic equivalence, as described by the EMA in its draft guidance, are numerous and should be selected depending on the specific features of each GI drug class. Therefore, to remain within the primary purpose of the present article, we have paid attention only to the recommendations concerning products acting locally in the intestine (Figure 1). In this regard, the EMA proposes that, in the case of modified release products with systemic bioavailability, such as 5-ASA, BE studies could be based on the assessment of drug plasma concentrations along with the estimation of partial AUC parameters, in order to discriminate the absorption resulting from an early drug release from that ascribable to drug release at the site of action. For these drugs, BE studies could be designed as single-dose PK tests under fasted and fed conditions, or as multiple-dose tests in the case of prolonged release formulations [64]. Partial AUC should be taken as the primary PK end point in each type of BE study. Of note, these approaches can be considered as surrogates for the equivalence of efficacy and safety profiles only if the systemic absorption of the active ingredient occurs at the site of action [64]. The implementation of a new regulatory framework for locally acting GI products has been waited for a long time. In this respect, the release of this new draft guideline represents a relevant regulatory advance in this area. It is likely that this new scenario will have a significant impact on pharmaceutical companies, promoting more generic competition through a clearer regulatory approach, conceived for streamlining the demonstration of therapeutic equivalence for locally acting GI drugs.

5. New evidence about the in-vivo GI behavior of 5-ASA medicinal products

Understanding the implications that the pharmaceutical features of locally acting drugs have for their in-vivo and clinical
performance is crucial for a correct definition of the BE criteria for such products. Several research and regulatory projects are ongoing in this field, as reported by the FDA [82]. With regard for 5-ASA, FDA started a collaboration with the University of Michigan focused on the BE challenges related to GI behavior of 5-ASA medicinal products [31,83]. To address this important issue, a clinical trial is ongoing [83]. The aim of the study was to measure directly the dissolution and release of oral 5-ASA formulations in the stomach, duodenum, jejunum, and ileum; to quantify 5-ASA content in the colon (through a measurement of its excretion in the feces); and to investigate and provide data to FDA about the relationship between the concentration–time profiles of 5-ASA in the plasma and GI tract [31,83].

The collection of GI fluid samples was accomplished by a new device consisting of a multi-port luminal catheter with fluoroscopic positioning of four gastric and small bowel aspiration ports, to quantify the regional drug concentrations throughout the digestive tract. The study was performed in accordance with a randomized, open-label, crossover PK design (modified release formulations), followed by a single-arm treatment (oral 5-ASA solution). Healthy volunteers were enrolled to receive one of the three modified release 5-ASA formulations: 2 × 500 mg of 5-ASA controlled release capsules, 3 × 375 mg of 5-ASA extended release capsules, and 1 × 1200 mg of 5-ASA delayed release tablets [31,83]. After the crossover phase, subjects received an oral solution containing 100 mg of 5-ASA. Such an immediate release 5-ASA arm was included to obtain baseline individual PK parameters to be employed for the deconvolution process and developing a new index designated as composite appearance rate (CAR) [31]. CAR was defined as the net appearance of 5-ASA into the systemic circulation and was conceived specifically to capture disintegration, dissolution, transit, and absorption of modified release 5-ASA formulations. Deconvolution is a predictive model that was used to dissociate the absorption phase from the clearance and volume of distribution that are involved in the systemic bioavailability of 5-ASA. In particular, the authors chose to isolate the absorption process for reducing putative bias due to inter-subject variability of the clearance and volume of distribution as well as to predict the physiological process involved in the appearance of the observed drug profile. According to this model, the baseline plasma profile, obtained upon administration of the 5-ASA solution, was used to calculate the CAR of 5-ASA versus the time of the three tested formulations. Based on these results, CAR was validated as a reliable composite index able to predict the absorption rate of 5-ASA from the GI tract [31,84,85].

The above clinical study documented for the first time an in-vivo sampling and comparison of GI 5-ASA concentrations released by three different formulations. The analysis of data highlighted a variable release of 5-ASA and its metabolite (acetyl-mesalamine) in the GI tract, in line with the technology involved in the development of the formulations administered in this study. By contrast, comparable fecal levels of acetylmesalamine were detected, suggesting similar profiles of dissolution and release at the site of action. Furthermore, relevant information were obtained about the in-vivo relationship between the modified release forms of 5-ASA and several parameters related to the GI system, such as solubility pH, GI transit, and motility that have been often regarded as unpredictable variables [31].

The results obtained from the application of CAR are also of great interest. Indeed, this index was able to reflect the dissolution and release of each formulation, generating final score values that were in line with plasma 5-ASA concentration and its in-vivo GI release. Each modified release formulation showed a specific CAR profile; therefore, this index was able to detect differences among the in-vivo GI behavior of the three formulations employed in the clinical study. Due to CAR performance, the authors have proposed this index as a potential new tool to improve the demonstration of BE for locally acting GI and modified-release products [31]. Of note, the above results will likely provide also a scientific basis for the development of more accurate methods suitable for BE testing of locally acting drugs, or to reevaluate the relevance of traditional BE methods, and to review and develop BE guidances concerning other medicinal products. In US, according to data published by the FDA, the outcomes of this study have been taken as a basis for the development and review of BE guidances specifically dedicated to oral modified release 5-ASA products, oral modified release budesonide products, and binding agents [13].

6. New perspectives on generic drug development and authorization

In recent years, the significant advances made in biopharmaceutical sciences, along with the BE issues associated with complex drug products, have fostered the introduction of new tools into the process of drug development. These new methodologies, such as modeling and simulation tools, will likely play a considerable role in the future perspectives of pharmaceutical and regulatory sciences, simplifying and reducing the failure rates of research and development procedures.

In this context, the physiologically based pharmacokinetic (PBPK) is one of the most widely used in the development of both generic and new drug products. PBPK is a mathematical model that integrates the physicochemical properties of drug substances, formulation properties of drug products and physiological parameters in order to predict in-vivo absorption, distribution, metabolism, and excretion [86–89]. Several applications of this model have been defined in the area of pharmaceutical sciences, and several research and regulatory activities are ongoing in this field [90]. For instance, the Orbito Project (Oral Biopharmaceuticals Tools) has been funded by the European Commission with the aim of expanding our knowledge about the interaction between orally administered drugs and the digestive system, and thereby developing new experimental approaches and simulation models that should allow of better predicting the in-vivo performance of drug products [91].

Pharmaceutical companies are increasingly employing the abovementioned tools to address BE issues related with drug products [91–95]. The application of the PBPK model is less complex in the context of generic drug development, due to the availability of PK and pharmaceutical data of the originator.
that could be used as input parameters or to calibrate and validate the tool [90]. According to FDA data, absorption modeling has been used by pharmaceutical companies in 34 medicinal products reviewed by the agency from 2010 to 2016 both for immediate and modified release formulations [96]. More interestingly, from 2009 to 2015, some label information for 26 approved medicinal products were extrapolated by means of the PBPK model [96]. According to the increasing use of this tool by pharmaceutical companies, both the EMA and FDA have released guidelines on the use and validation of PBKP during the development of new drug products [97,98].

In the context of regulatory activities, since 2004, the FDA Office of Generic Drugs has adopted the above tools for better characterizing several equivalence issues in about 22 projects that included the development of BE guidance, revision of the abbreviated new drug application, regulatory research studies, and post-approval issues [90]. With specific regard to BE for oral 5-ASA products, the FDA applied the simulation and prediction models in order to support the use of AUC

$\int_{t_{1}}^{t_{2}} \frac{AUC_{1}}{AUC_{2}}$ as a PK parameter that is able to better correlate, than other partial AUC parameters, with the colonic exposure to the active ingredient when two 5-ASA extended release capsules were compared [99].

### 7. Conclusions

The advances made in understanding the scientific features of locally acting GI drugs has led to considerable progress in the BE area. Regulatory agencies have released guidelines dedicated specifically to locally acting drugs to provide advices and simplify the development of generic medicinal products. However, a remarkable difference can be noted when comparing the EU and US regulatory activities in this field. The FDA has delineated a specific regulatory framework with the development of product-specific guidances that define a clear strategy for each product belonging to this pharmacological class. In the case of oral modified release 5-ASA products, partial AUC was identified as a new PK parameter that allows to correlate drug plasma profile with its local concentrations at the site of action. Furthermore, the FDA has started a number of additional research initiatives to provide a strong scientific basis for locally acting GI drugs and promote further the scientific and regulatory advances for such products. In Europe, the regulatory framework has been characterized for 20 years by a general paradigm that was difficult to apply to all types of drug products belonging to the class of locally acting GI medicines. However, very recently, with the publication of the draft guidance that revises the guideline issued in 1995, the EMA is trying to change the European regulatory framework pertaining to the demonstration of therapeutic equivalence for locally acting GI drugs, moving toward a new regulatory framework that reflects closely the one adopted by the FDA. This shift denotes a great advance in this field, as it opens to a simplification of the scientific and regulatory process that pharmaceutical companies should follow to achieve marketing authorization. The possibility of demonstrating therapeutic equivalence through in vitro and/or PK BE studies, rather than comparative clinical and PD endpoints, will likely result in a significant impact on pharmaceutical companies, which are implementing research and development programs to foster competition in this field. In this light, it is desirable that the new EMA draft guidance will be quickly approved and made available in its definitive.

### 8. Expert commentary

BE studies play a key role in the development of generic drugs and new drugs. For most systemically absorbed drugs, the scientific and regulatory framework is well established and conventional PK studies still represent the gold standard approach to demonstrate BE. However, for more complicated drug products, there are still many open questions. For locally acting GI products, the development of a regulatory strategy has been complex and it has been often associated with uncertainties related to scientific and regulatory aspects. The lack of specific BE regulatory recommendation has generated a negative impact on the market competition reducing economic savings for both patients and National Health Services due to the high cost and risk of failure associated with the clinical development process [11,82]. The case of oral modified 5-ASA drug products summarizes the challenges when considering the development of a BE strategy for locally acting GI drugs. The suitability of clinical efficacy studies or PK studies for assessing BE of oral 5-ASA formulations has been matter of debate, with much discussion revolving around their potential correlation with local concentrations achieved by 5-ASA at the site of action, or even its release behavior from oral formulations throughout the GI tract [8–12]. Several additional factors, such as intra-subject variability of 5-ASA plasma concentrations and the influence of physiological GI parameters, amplify further the degree of complexity and increase the risk of failing BE demonstration [31]. However, thanks to advances that have been made in this area, the BE recommendations for locally acting GI drugs have substantially changed over the last 10 years, as seen by the evolution from clinical end point BE studies to tailored experimental approaches able to ensure a sensitive and accurate detection of putative in-vivo differences between the test and originator product. The introduction of partial AUC as PK parameter that allows to correlate 5-ASA plasma profile with its local concentrations at the site of action is one of the interesting advance that has been made in this field. However, the activities implemented by EU and US agencies in this context differ in several respects. Improvements have been introduced, both from a scientific and regulatory standpoint, by the FDA, with the development of specific guidances and the promotion of new research activities. By contrast, for long time, only general recommendations have been issued by the EMA and other European National Agencies. Only in April 2017, with the publication of the draft guidance that revises the guideline issued in 1995, the EMA is trying to change the European regulatory framework pertaining to the demonstration of therapeutic equivalence for locally acting GI drugs, moving toward a new regulatory framework that reflects closely the one adopted by the FDA. This shift denotes a great advance in this field, as it opens to a simplification of the scientific and regulatory process that pharmaceutical companies should
follow to achieve marketing authorization. This new scenario will likely result in a significant impact on pharmaceutical companies, promoting more competition through a clearer regulatory approach, conceived for streamlining the demonstration of therapeutic equivalence for locally acting GI drugs.

9. Five-year view

In recent years, the increasing advances in the knowledge of pharmaceutical and pharmacological features of locally acting drugs have led to improvements in the processes of BE evaluation, thereby fostering the development of alternative BE methods, able to document with high accuracy similarity profiles and in-vivo differences between different medicinal products. The regulatory paradigm has been largely modified, and considerable improvements have been made both from a scientific and regulatory standpoint. This new scenario will simplify the scientific and regulatory process that pharmaceutical companies should follow to achieve marketing authorization. However, since BE is a continuously evolving science, we can speculate that simulation and prediction tools will likely play a considerable role in all phases of the research and development of generic drugs. The full implementation of these models in the future perspectives of pharmaceutical and regulatory sciences will help to demonstrate the therapeutic equivalence for complex drugs as well as to assess potential failure modes and risks to product equivalence, simplifying the research and development procedures.

Key issues

- Bioequivalence testing for locally acting gastrointestinal drugs is a challenging issue for both regulatory authorities and pharmaceutical industries. The international regulatory framework has been characterized by the lack of specific bioequivalence tests that has generated a negative impact on the market competition due also to the high cost and risk of failure associated with the clinical development process.
- Thanks to advances made in this field, novel bioequivalence criteria have been recommended by regulatory agencies and several research activities have been promoted to develop alternative methods suitable for detecting putative differences in the in vivo behaviour of locally acting gastro-intestinal drugs.
- In April 2017, the European Medicines Agency has published a draft guideline, which proposes a new scientific and regulatory paradigm that simplifies the demonstration of bioequivalence for locally acting gastrointestinal drugs.
- Partial area under the curve, that allows to correlate systemic bioavailability and drug concentration at the site of action, is a key pharmacokinetic parameter for the BE assessment of oral modified release mesalamine formulations.
- Combination of comparative in vitro dissolution tests and properly designed PK studies, under fast and fed condition, are suitable for detecting any difference in the drug release, and estimating if two 5-ASA products are absorbed at the same rate and extent at the site of action.
- Modeling and simulation tools will likely play a considerable role in the future perspectives of pharmaceutical and regulatory sciences, simplifying and reducing the failure rates of research and development procedures.

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Declaration of interest

G Sferrazza has no conflict of interest to report. PD Siviero has served as a speaker for Roche, a consultant for Roche, Biomarin and Boehringer Ingelheim, an advisor board member of Janssen Cilag. G Nicotera has no conflict of interest to report. P Turella has served as a consultant for Sofar. A Serafini has no conflict of interest to report. C Blandizzi has served as a speaker and consultant for Alfa-Wassermann and Sofar. P Pierimarchi has no conflict of interest to report. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Papers of special note have been highlighted as either of interest (+) or of considerable interest (+++) to readers.

This book chapter provided an excellent overview of the bioequivalence and clinical aspects related to locally acting gastrointestinal drugs.


This paper showed the challenges involved in the development of generic drugs focusing on scientific and regulatory aspects that could accelerate the approval of new drug.


Review on mesalamine use in ulcerative colitis.


72. In these citizen petitions, the FDA introduced the partial AUC as pharmacokinetic parameter able to correlate mesalazine plasma profile with its local concentrations at the site of action.


79. European Medicines Agency. Concept paper on the development of additional regulatory documents that should be followed for the development of different type of local acting drug products.