Defining drug purity through chromatographic and related methods: current status and perspectives

A.M. Krstulovic*, C.R. Lee

Analytical Chemistry Group, Synthélabo Recherche, 1 Avenue Pierre Brossolette, 91380 Chilly-Mazarin, France

Abstract

Chromatographic and electrophoretic techniques play a preeminent role in assessing the quality of drug substances and drug products. Current ICH guidelines place in a legal framework what has been common practice in modern pharmaceutical research and quality control. This paper reviews some aspects of current requirements for evaluating the purity of chemically synthesized new drug substances and drug products, and suggests some possible future trends.

Keywords: Drug purity; Drugs

Contents

1. Introduction

Purity is a metaphysical concept; absolute purity does not exist and the degree of purity of a product is only a reflection of the analytical techniques used for its assessment. All pharmaceutical substances unavoidably contain impurities and the role of ethical pharmaceutical industry is to define an impurity profile that is acceptable for the intended use of a given drug, without compromising its therapeutic safety and efficacy. Purity of drug substances and drug products is a subject which can constantly be revisited and notions have been evolving over the years. While purity has always been considered as an

*Corresponding author.

PII S0378-4347(96)00439-2

Although this results in a much better appreciation of drug purity, the analytical chemist is still haunted by the unpleasant thought: "Have I seen everything?" The answer to that is, of course: "No, since for example, HPLC with UV absorbance detection could be replaced by HPLC–MS, the classical pharmacopoeial heavy metal test by inductively coupled plasma-MS, etc". The role of the analytical-pharmaceutical chemist is to decide what information is essential for release, to select appropriate analytical methods and to set justified and realistic specifications. Beyond this point all additional findings, however interesting, can be considered as intellectual curiosities.

Defining limits for impurities is more than an exercise in toxicology. For example, although the limit for organic impurities in active ingredients (0.1%) above which action is required under ICH guidelines [1,2] may have been based primarily on information held by regulatory authorities concerning past cases of adverse reactions, some other reasons for choosing 0.1% could be: (1) to ensure consistent assay values for drug substances; (2) to define the level of performance that the regulatory authorities expect of analytical laboratories and (3) to limit the scope for variations in the manufacturing process, the only practical precaution that can be taken against the risk of unexpected adverse reactions (such as allergy) that may be caused by trace impurities.

Limits for particularly toxic impurities such as alkylating agents and other potential carcinogens seem to be determined more by the analytical possibilities than by considerations of safety margins. A case in point is the limit of 2 ppm set for benzene: this may seem unduly conservative bearing in mind that one breathes the vapours of a 5% solution when filling one's petrol tank. However, it ensures that benzene is not used in the manufacturing process, it sets performance criteria for the GC equipment (which is relatively less sensitive to some other solvents such as chloroform), and, last but not the adoption of more stringent criteria seems unlikely in the foreseeable future. While it might, therefore, be presumed that current analytical methods are adequate, there are still one or two gaps, and economic and environmental aspects have to be considered. Drug products present more complex problems due to their inherent instability, and much remains to be done, not so much on the analytical side as on setting specifications.

2. Drug substance

For a drug substance synthesised according to a well-defined synthetic route, physico-chemical analyses carried out during release must guarantee three essential acceptance criteria: identity, purity and assay. These criteria concern both the chemistry and the physico-chemical state of the drug substance. It is the combination of all the test results that permits a decision to be made concerning the acceptability of the drug.

2.1. Identification

According to current practice, identity tests are carried out on all individual containers, while purity tests and the assay are carried out on an average (pooled) representative sample. The latter sample is considered as having legal value and as such, enough of it should be conserved for four additional complete analyses in case of disagreement.

In the case of salts it is essential that both the pharmacophore and the counter-ion be identified. Although some pharmacopoeias favour colour reactions requiring little equipment, research and development companies should apply the full range of routine chromatographic and spectroscopic techniques to each batch. IR spectroscopy is the most useful and commonly used molecular fingerprinting technique, since besides giving structural information and rigorous proof of identity, it often detects

If the drug substance is to be used in solid dosage forms, it is most important to identify and quantify the polymorphic composition of each batch, since this can have an impact not only on bioavailability, but also on the physical properties of a drug (e.g., hygroscopicity) and hence on the manufacturing process. During drug development, as soon as enough of a new drug substance becomes available, attempts are made to provoke the formation of different polymorphic forms by recrystallising the compound from different solvents at different temperatures and by subjecting it to thermal and mechanical stresses. It is important to carry out this study early, since once a new polymorph appears it may not be possible to go back to one observed previously. Polymorphism can be studied using either non destructive methods, such as microscopy, X-ray diffraction, IR spectroscopy and Raman spectroscopy, or destructive methods, such as differential thermal analysis and thermomicroscopy.

Powder X-ray diffraction analysis is essential at the development stage, as neither thermal analysis nor IR spectroscopy (which relies on second-order effects on vibrational transitions) can be relied upon to reveal all cases of polymorphism. Besides being a definitive method, X-ray diffraction gives results that are relatively clear-cut and easily interpreted. It should be noted that the sample to be examined should contain particles of the order of several micrometers in order to minimise the effects of preferential orientation. Larger particles should be ground, which introduces the risk of changing the composition by pressure or heat. In the case of solvates, solvents can be lost or taken up by the solid. As an example from our laboratory, Fig. 1 shows the distinctly different X-ray diffractogram of 3 polymorphs of a drug substance: characteristic lines can conveniently be exploited for quantitative analysis. The expense of the equipment may preclude use of the technique in routine production environments, but fortunately IR spectroscopy is often found to be adequate for the quality control of transformation. In these cases, the diffuse reflection technique is useful since it is carried out directly on the powder. Fig. 2 shows the diffuse reflection IR spectra of the three polymorphs illustrated in Fig. 1.

Thermal analysis is an important set of techniques (melting point determination, thermogravimetry and differential thermal analysis), because it can give information on the relative stabilities of polymorphs and can also reveal differences in physico-chemical properties, such as degree and nature of solvation. In some cases, quantitative analysis is possible. Fig. 3 shows the DSC-TGA curves of the three polymorphs whose diffractograms are shown in Fig. 1; one of them is hygroscopic, as demonstrated by the broad endotherm at 121.15°C (corresponding to a 4.92% loss in mass). Coupling of TGA with IR spectroscopy or MS makes possible the identification of the volatile products responsible for the mass loss.

Granulometry is an important factor which should be carefully followed particularly for drug substances of low solubility, since it will have an impact on drug bioavailability. In these cases, specifications should be established. Specifications are not needed for soluble drug substances, although this information may be of interest, for example, for ensuring content uniformity of the corresponding drug products. In this case, granulometry becomes a manufacturing specification of the drug product.

2.3. Impurities

Only chemical impurities are considered here, although microbiological impurities are also important in many cases. Their profile is influenced by: the choice of synthetic route, the quality of starting materials, reagents and solvents, the reaction conditions, the work-up and final purification, as well as the design of process equipment. Thus, analytical development must go hand-in-hand with process design. Additional impurities may arise from degradation during storage or extraneous contamination, although the latter is a Good Manufacturing Practice

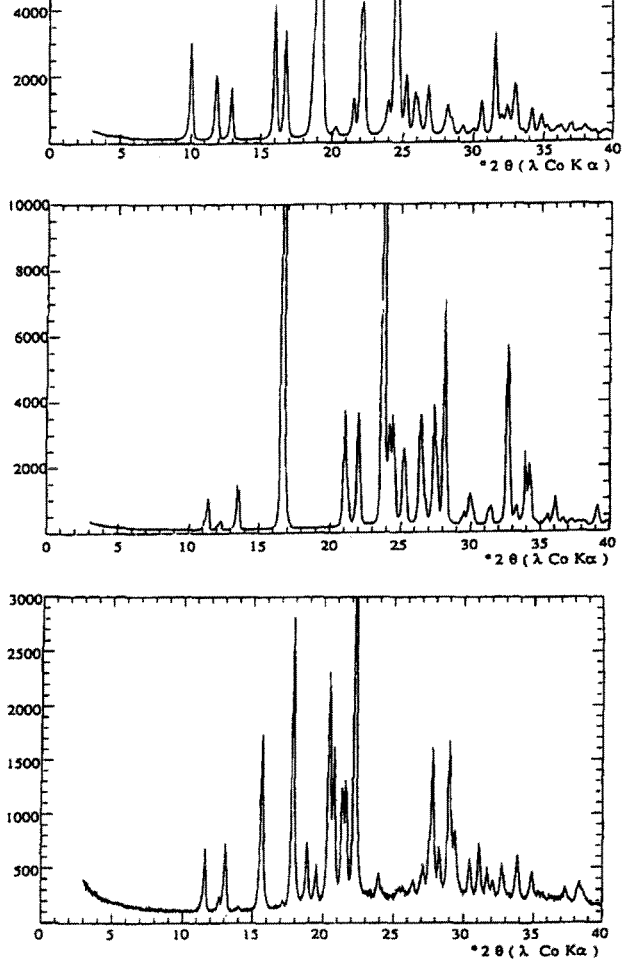

Fig. 1. X-ray powder diffraction patterns of 3 polymorphs of a drug substance. Apparatus: Philips PW 1710; X-ray tube: λCoKα, 40 kV, 40 mA.

(GMP) issue which will not be discussed further here.

Chemical impurities are classified for regulatory purposes as organic, inorganic and residual solvents.

Organic impurities can originate from impurities contained in starting materials (most often isomeric impurities), synthetic intermediates (incomplete reaction or excess reagent used) and degradation products which may depend on alterations in reaction conditions, such as temperature, pH, or in storage conditions (hydrolysis, oxidation, ring opening, etc.). For an identical synthetic route, scale can have an influence on the impurity profile, since transport phenomena, heating and cooling efficiency, mixing properties and residence time at various stages are different. Nearly all organic impurities are determined by chromatographic or related methods of which HPLC has been the most important for well

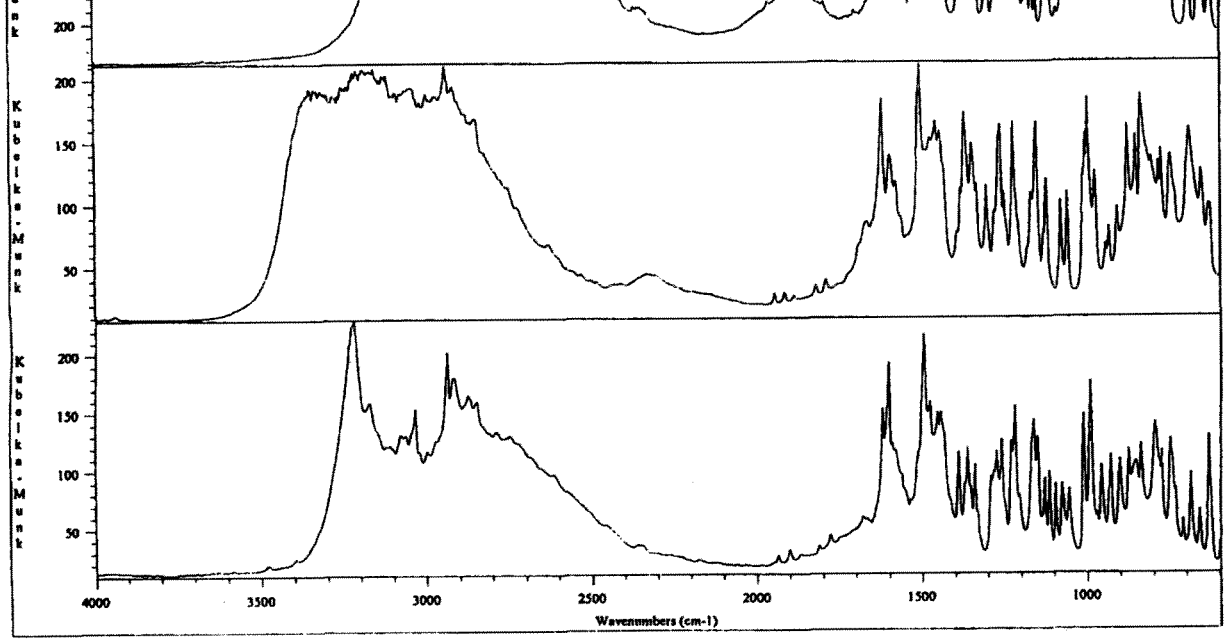

Fig. 2. Diffuse reflection IR spectra of 3 polymorphs of a drug substance. Apparatus: Nicolet, Magna-IR 500 Spectrophotometer with Spectra-Tech diffuse reflection accessory.

over a decade. When compared with some other techniques, the chromatographic efficiency appears modest, but this is compensated by the possibilities for varying retention and selectivity. Most workers use the reversed-phase mode with UV absorbance detection whenever appropriate, because this provides the best available reliability, analysis time, repeatability and sensitivity; the technique, in fact, sets the standard against which others are compared.

Generally speaking, gradient elution, although extensively used in pharmaceutical research, is not popular in quality control, because many of the above advantages are lost. Instead, screening for potential impurities is often performed by a combination of isocratic HPLC methods. For example, the search for 11 potential impurities of mizolastine requires the use of three isocratic HPLC methods (Fig. 4a–c) because of the large differences in the hydrophobicities of the impurities. Whenever possible, the levels of impurities originating from the starting materials should be limited through appropriate in-process controls in order to avoid the need for their monitoring in the drug substance. Thus, for example, the assay for isomeric impurities of mizolastine may not be necessary if sufficiently stringent specifications are set for in-process controls. Limitations of HPLC include the cost of columns and solvents and a lack of long-term reproducibility due to the proprietary nature of column packings. Moreover, for analytes that are not detected by the UV absorbance detector, there exists no alternative having the same combination of performance characteristics. HPLC may, therefore, be complemented by GC, TLC or CE, and in some cases by tests not involving a separation method.

The range of analytes amenable to analysis by GC is too limited for this ever to become a major chromatographic technique in most areas of pharma-

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.