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Analytical Survey

Purity requirements from a pharmacopoeial point of view*

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Abstract: The nature of pharmacopoeial specifications for bulk drug substances 25–30 years ago and the analytical methods used at that time are briefly reviewed. Since then the concept of characterizing drug substances by controlling impurities has been developed in pharmacopoeias. This concept is examined in relation to: pitfalls in practice; the precision and specificity of analytical procedures; the purity of many drugs nowadays; the usefulness of assays of drug substances; specifications for drug substances for which new methods of synthesis have been used; control of impurities in formulated products; and the significance of purity of drug substances and formulated products.

Keywords: *Drug purity; pharmacopoeial standards; impurities.*

Introduction

An interesting change has occurred in the nature of specifications for bulk drug substances in pharmacopoeias during the last 25–30 years. In those earlier times the traditional method for assessing the quality of a substance would be to identify the product and then to carry out an assay of some kind. These two major requirements would be supported by evaluation of other characteristics that were thought to contribute towards knowledge of the purity of the substance — for example, the determination of a melting point or of an optical rotation. Such tests would be augmented by one for heavy metals (or perhaps a specific test for lead), for sulphated ash to detect inorganic contamination and probably tests for the presence of anions such as chlorides and sulphates that, if present in undue amount, might be regarded as evidence of unsatisfactory cleaning-up of the finished material.

Consider briefly the analytical methods most widely used in the pharmaceutical analytical laboratory at that time. A reliable way to do this is to glance in the more well-established pharmacopoeias of that day. For example, by looking at the USP XVI, methods of assay based on classical titration techniques and on non-aqueous titrimetry are well in evidence, as are methods based on either direct UV spectroscopy or on a

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spectrophotometric method following colour development. Occasionally, as for digitoxin, a liquid chromatographic method was employed based on gravity elution at atmospheric pressure. The contemporary British Pharmacopoeia (the edition of 1958) shows interesting developments from the earlier edition (1953). For example, pethidine hydrochloride was directly titrated with silver nitrate in acid solution in the earlier edition whereas an extraction followed by non-aqueous titration was used in 1958. There was, in the British Pharmacopoeia, a rather more determined use of gravimetric methods than in the USP whereas the latter favoured rather more reliance on direct UV spectroscopy. These methods, for the most part rather non-specific, were reinforced by identification reactions where much reliance was placed on colour development, UV spectroscopy and derivative formation followed by melting-point determination. In the USP XVI there was a sprinkling of identification requirements based on infra-red spectroscopy with comparison against a reference standard. A number of monographs in those pharmacopoeias contained tests for specific named impurities or groups of impurities such as pseudo cyanocobalamin in cyanocobalamin, aglucones in ouabain or related foreign steroids in hydrocortisone. Such tests were largely based on physical or chemical attributes; however, for related foreign steroids, a paper chromatographic method was invoked in the USP XVI although not in the British Pharmacopoeia until 1963. Those analysts who may have engaged in the paper chromatography of steroids using either the Bush or Zaffaroni systems will know that these systems did not provide the slickest of answers but at least the concept was beginning to be developed that the trace impurity pattern of a bulk drug substance might, in many cases, tell a lot more about the purity of the product than any number of assays would do.

But why only paper chromatography to achieve this control of impurities? To answer this question one needs to recall that gas chromatography was demonstrated for the first time in 1952 and that for the first several years of its life its practical usefulness was confined to volatile liquids such as those of concern to the petroleum industry. The BP 1958 would have been sent to the printer late in 1956 and even the USP XVI would have been completed by late 1958 or early 1959; the principles of gas chromatography were just not being applied in a pharmaceutical sense at that time. One further needs to remember that the first demonstrations of the power of thin-layer chromatography were not undertaken in Western Europe until 1956 and onwards and if one looks into United States literature one will find that the first papers describing the technique and some of its applications did not appear in Analytical Chemistry until the early 1960s. Liquid chromatography as it is known today was still very much a thing of the future.

Characterization of drug substances by controlling impurities

As these techniques became better established and more versatile in their application the concept of characterizing a bulk drug sustance by controlling what should not be present rather than what should be present began to emerge and was developed, it is probably fair to say, at a somewhat faster rate in Europe than in the United States.

The concept rests on the belief that, for a bulk drug substance, what is needed to give an assurance of acceptable quality is first to identify the material in an unequivocal way and second to demonstrate that the substance is essentially homogeneous. Once these two factors are established there seems, to some, little point in requiring an assay to be carried out. It might be argued that if the material has been identified as that required and if a series of tests then give reasonable assurance that there is no more than, say,

0.5% of total impurity present, what else can the sample be but a 99.5% pure product? Why then carry out an assay procedure that, because of the analytical tolerances required due to imprecision of the method, might allow a judgement as to whether the material contains somewhere between 98.0 and 102.0% of the required entity?

This over-simplification of the overall power of the approach of controlling materials through impurities is, of course, somewhat naive. It is necessary to examine the concept in much closer detail to see where the possible advantages and pitfalls lay. Nevertheless it does underline why purity requirements from a pharmacopoeial point of view depend so much on determination of impurity levels.

Pitfalls in controlling impurities in drug substances

Assume that a material has been correctly and adequately identified. According to my premise, the next most important characteristic to determine is the quantity and hence the nature of each trace of material present that is not of the same chemical entity (or in some cases not of the same physical character) as the main bulk. This, of course, is a difficult and demanding task. It might be expected that the manufacturer of a bulk substance, knowing the synthetic route used, should adequately characterize the nature of all extraneous components. It will be recognized that it is essential to know the identity of trace impurities before it is possible to assign a truly quantitative value.

For separation and quantitation of impurities much reliance is now placed on chromatographic methods. Unfortunately a single chromatographic procedure might be quite inadequate to fully separate all impurities that could occur. Analysts are all familiar with those materials that may stay at the point of application or the point of injection and are also well aware of those closely related compounds that might be eluted under the umbrella of the main ingredient. In complex situations it may be necessary to use two or three different systems of chromatography to expose all potential impurities; an example is the British Pharmacopoeia monograph for ibuprofen, where there are two gas chromatographic requirements and one thin-layer chromatographic requirement.

In addition to these problems it has to be recognized that other impurities may be present that would not be detectable by such chromatographic systems, for example, inorganic salts. For this reason a number of additional tests of a standard nature will be required to give an assurance on this point.

Precision and specificity of analytical procedures

Despite the possible problems that have been indicated, however, I believe that the approach of unequivocal identification followed by demonstration of homogeneity is usually the soundest way to assess the real quality of a bulk drug substance. One of the major contributing factors to this belief stems from my life-long concern with the precision of analytical methodology. The fact is that all analytical procedures are subject to imprecision in greater or lesser degree. I do not subscribe to the view of clever statisticians who can work out theoretical tolerances for assay procedures that would make them totally unacceptable. I believe that tolerances should be established in an empirical way rather than a theoretical one and should be based on results actually obtained in practice by experienced technicians working, over the years, on substances of acceptable quality. As a generalization (although there are certain notable exceptions) the more specific a method is made the greater its imprecision. A favourite pharmacopoeial method of determining the 'purity' of many bulk pharmaceuticals is non-aqueous titrimetry; this is probably capable of being one of the most precise

analytical methods but it is certainly very non-specific. To endow it with specificity it is often necessary to introduce various additional stages to an assay procedure and each stage introduces its own quota of imprecision. Even this very precise method is being taken away from the analyst now because of an almost neurotic concern at the use of mercury salts on the part of some people. I am convinced that the replacement methods that are being proposed are already less precise than those they are replacing.

Purity of bulk drug substances

To set against these imprecisions one has to recognize nowadays that bulk pharmaceuticals are of such purity that variations between what might be regarded as an excellent sample and what might be regarded as a poor sample are likely to be less than must be allowed for variation due to imprecision of the analytical method. As an example consider the USP XXI monograph for clofibrate. As currently manufactured by major producers, this is a very pure material that contains only traces of other volatile related substances and possibly a very low level of free phenolic materials. Yet, because the USP authorities were anxious to apply an assay, based on passage through an ion-exchange column followed by spectrophotometric measurement at 226 nm, the tolerance limits given at the head of the monograph are that "Clofibrate contains not less than 97.0% and not more than 103.0% of $C_{12}H_{15}C10_3$, calculated on the anhydrous basis". The casual observer looking at this monograph might be forgiven for supposing that clofibrate was a rather impure material containing two or three per cent of impurities. In fact these wide tolerances are simply there to accommodate the inadequacies of the assay procedure. It would surely be better not to give an assay at all but to rely on sensitive tests for free phenolic materials and for volatile related impurities by, for example, gas chromatography. This would, in my opinion, give a much better evaluation of the real purity of the clofibrate. Just to demonstrate that I am prepared to put my money where my mouth is, look at the monograph for clofibrate in the British Pharmacopoeia.

But, one will no doubt say, many of the chromatographic procedures that might be applied to determine the minor constituents of the bulk drug substance are themselves subject to imprecision, often many times greater than would be the case for the formal assay. This is, of course, quite true. A thin-layer chromatographic test based on visual assessment probably has, at best, a plus or minus 20% chance of hitting the target. Use of instrumental techniques such as scanning densitometry may reduce this imprecision by about half. Consider the implications of this. Suppose the impurity is, in reality, present to the extent of 0.5%. The imprecision of the technique, if one accepts what I have just said, would mean that a judgement might be made that the impurity is present at somewhere between 0.4 and 0.6%. If this could be demonstrated to be the only impurity present then the conclusion might be reached that the required molecule in the bulk substance must be present to the extent of 99.4–99.6% — a very much better assessment than even non-aqueous titrimetry could afford.

There are problems in this approach, as have been indicated. For example, I can call to mind a particular benzodiazepine where degradation may lead to the presence of an impurity which, by the most readily available methods of detection after chromatography, reacts at a sensitivity that is about 2½ times that of the substance being tested. If, therefore, as is commonly the case, particularly with pharmacopoeial procedures for thin-layer chromatography, comparison is made with a loading of the bulk substance itself (the so-called high–low technique), quite misleading results might be obtained. By the time the degradation product has become present at a true level of about 1% it might

be estimated as being there at a level of 2½%. More seriously, there are undoubtedly other cases where a gross under-assessment is possible. Clearly this kind of misleading problem must be investigated and compensated for when the method is being established.

To make the general concept foolproof it is necessary to have identified every impurity since only after identification can a quantitative estimation be confidently established. It is also necessary to be confident that the separative method or methods used will indeed reveal all likely impurities.

Usefulness of assays of drug substances

Despite my strong plea for principal reliance in assessment of purity on the determination of impurity levels and my feeling that the application of an assay might sometimes be misleading, I think that an assay is nevertheless useful for the majority of substances. For example, a drug substance might be 'cut' with 5% of lactose and this would be unlikely to be recognized during identification by, for example, infra-red spectroscopy and also would be unrecognized by, for example, a liquid chromatographic separation using UV detection. Most assays, however non-specific, are likely not to respond to lactose; a notable exception might be the tetrazolium assay commonly applied to corticosteroids. The assay also serves, in many cases, to give an added assurance of identity. For example, a drug substance susceptible to non-aqueous titrimetry, if it assays at 99–101%, may be assumed to have approximately the right molecular weight. The titration, however, adds little to assurance of the purity of the substance. The assay should therefore be viewed in proper perspective; it is often the least significant part of the total specification. In this context I believe that the time-honoured presentation of monographs in pharmacopoeias is itself misleading; an opening statement, usually in a larger size of type than is used for the rest of the monograph, might declare, for example, "it contains not less than 98.5% of $(C_{17}H_{23}NO_3)_2$, H_2SO_4, calculated with reference to the dried substance". This statement applies to atropine in the European Pharmacopoeia and the assay procedure applied there is a non-aqueous titration. In the European Pharmacopoeia the type size is actually uniform with the rest of the monograph but in the British Pharmacopoeia the statement is in large type as it is in the USP. In the USP even more attention is drawn to the statement by that symbol which always reminds me of a US army corporal lying down with his jacket on; this makes it appear to be the most important statement in the monograph. I would submit that far more important for assessment of purity is the unequivocal identification and compliance with such requirements as the chromatographic procedure for foreign alkaloids that the USP (but not the European Pharmacopoeia at the present time) contains, together with a test for optical rotation and possibly for melting range.

So far I have said little about the power of liquid chromatography. Here, perhaps for the first time, we have a fairly generally applicable method that is capable of both specificity and an acceptable degree of precision. Increasing use of liquid chromatography as an assay procedure that would give greater confidence of the purity of a drug substance is thus to be expected. It may be, however, that the same method, suitably adapted, might be used to quantitate individual impurities and here we are faced with a dilemma. Conceivably also one could use the same liquid chromatography as a means of identification by use of comparative elution times. We would then have the situation that a single method could be used to identify, test for homogeneity and assay. This versatile method could thus save a great deal of time in sample examination and I have actually

heard it proposed that specifications of the future might be developed in this way. It must be very evident to any thinking scientist that such a reliance on a single technique would be totally unacceptable. The essence of purity assessment in a pharmacopoeial specification is the interlinking and complementary nature of various different tests. Only if all tests are satisfactorily met by the sample can it be considered to be acceptable. If the various tests are based on a variety of different techniques this will give a much more confident assurance of the quality of the material. It is for this reason that I would counsel against too extensive a use of liquid chromatography for assay purposes. In my view the power of that technique is best harnessed to examination for impurities.

Much is spoken about stability-indicating assay proceduress. These are absolutely essential for the examination of formulated products but in applying them due account must again be taken of the likely precision of the method. In the event that degradation is fairly marked under certain storage conditions then a truly stability-indicating assay may be used. By definition, however, this would have to have a considerable degree of specificity for the intact molecule and, for reasons earlier stated, this specificity may have to be bought at the expense of increased imprecision. If the degree of degradation is small, however, but nevertheless important to detect, then a stability-indicating assay, as such, may not be appropriate. This is certainly the case, in my view, when the stability of bulk drug substances is being considered. Thus an increase from 0.2% of the particular impurity to 0.6% within a short period would represent a serious rate of degradation but the fall in specific assay value of the intact molecule from 99.8 to 99.4% might well be ascribed to analytical variation from one occasion to another. Again the possible exception to this concern might be offered by liquid chromatography, particularly with the advent of diode array detection systems.

Specifications for drug substances synthesized by new routes

From a pharmacopoeial point of view there is always a theoretical, sometimes a little more than theoretical, possibility that a well known drug substance for which a long-established and reliable specification has been developed may become available from an alternative source that perhaps is employing a hitherto unencountered method of synthesis. Once such a source of material has been recognized it must be incumbent on the pharmacopoeial authority to determine whether or not the existing specification is adequate to control material from the new synthetic route; should the specification be found wanting an appropriately revised monograph should be issued without delay. The success of any particular pharmacopoeia in dealing with such a situation must depend upon the speed, or lack of speed, with which it can react when a revision is considered necessary. Unfortunately, it must be admitted, in some cases this is all too slow.

Control of impurities in formulated products

I have said much about impurities and the need for their detection and control in bulk drug substances. The need for similar examination in formulated products is debated, however. This particularly applies to impurities that could only arise from synthesis of the active ingredient and that would thus not be expected to change during formulation or storage of the finished preparation. Some will say quite rightly that if an impurity arising from the synthetic process is determined and controlled in the original active ingredient there should be no need to seek that same impurity again in the specification for a preparation made from it. This is certainly true for a manufacturer of the final product since he will know exactly what batch of material was used in its preparation and

he will thus have the necessary information to hand. The purity of such a preparation from a pharmacopoeial point of view is, however, a different matter. The pharmacopoeial specification, designed as it is to apply throughout the accepted shelf-life of a product, may be used by analysts who have no knowledge or guarantees concerning the active ingredient that was employed. The examination of pharmaceutical dosage forms for such impurities may thus give a valuable insight into the quality of the active ingredient that was used — an insight that might not be achieved in any other way.

Significance of impurities in drug substances and formulated products

We are left with a final problem. How pure should an acceptable product be? What is the significance of traces of impurities not hitherto encountered? Should they be regarded as undesirable unless proven to be significantly non-toxic? Or should the alternative view be taken? All these are problems to which definitive answers are rarely available. Perhaps the best rule of thumb is to consider that any product admitted to the market should be at least as pure, or free from impurities, as the material on which toxicological and clinical work was first carried out. This is a problem for debate by toxicologists and clinicians.

As an analytical chemist my concern now rests with the sensitivity of methods for detecting these impurities. Some will remember the earlier days of application of chromatographic techniques when materials were referred to as being 'chromatographically pure'. Application of this ridiculous statement to a particular material would then be very quickly shown to be untrue as the limit of detection was lowered. Nowadays one must surely recognize that nothing can be regarded as 'pure'. That which seemed to be pure yesterday can be shown to contain impurities today and those materials which have so far defied detection of impurity will, I suspect, yield their purity up to research analysts in the coming years. One can recall delightful concepts from former years such as 'as pure as a snowflake' or 'as pure as the mountain air'. A Swiss investigator was able to demonstrate the presence of some 40 or 50 different volatile components in the fragrant mountain air sampled in the Alps in spring time.

Acceptable levels of impurities

Because of the tremendous power of our analytical techniques there is a great responsibility placed on those who have to determine the acceptable levels at which impurities might be present. The keen scientist might simply want to apply limits that correspond to the sensitivity of his methodology; this has to be guarded against at all costs. I have no doubt that many of the bulk drug substances that we use today could be prepared in purer form if this were insisted upon; but to what end? No tangible benefit to the health of the patient but a very noticeable effect on his pocket. Certain impurities, of course, can be recognised as highly toxic and must be limited as stringently as possible. An example of this might be tetrachloroparadioxin in hexachlorophane. But such examples are few in number and in the majority of cases the impurity pattern in the drug substance simply serves to demonstrate that Good Manufacturing Practice has been maintained and that the batch of material being tested is similar in nature to previous batches. It is thus incumbent on us to control our analytical enthusiasm for the methods at our disposal and to set requirements at levels designed to serve both the public health on the one hand and industrial economics on the other.

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