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Purity determination and evaluation of new drug substances*

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Abstract: As a negative concept, purity is the absence of impurities and, hence, is not measurable directly. There are two major approaches to the determination of the purity of a chemical compound. The first is the absolute approach, based on thermodynamic methods; the total amount of impurities can be determined without detailed knowledge of these impurities. The second is the chromatographic approach, which gives information about the detection and determination of impurities as far as their nature and chemical behaviour are known.

Impurities may be derived from different origins. Synthesis precursors, intermediates and side-reaction products are intrinsic impurities. In addition decomposition products, unwanted isomers and polymorphs may appear as impurities.

The evaluation of impurity levels is the basis for specifications of drug substances. A total proportion of impurities of less than 1% seems to be a reasonable goal. Identification of impurities present in proportions estimated to be 0.1% and above is also required. Finally, the setting of specification limits depends on a combination of factors based on knowledge of the chemistry and pharmacology of the components and the economics of the process.

Keywords: *Purity; determination and identification of impurities; origin and limits of impurities.*

Introduction

Because of the breadth of the subject, this article comprises an arbitrary selection of the many topics which could be presented. Consideration is given to the absolute and chromatographic approaches to the determination of purity of a drug, to the nature and significance of impurities, and to limits for impurities.

Absolute Approach to Purity Determination

An example of this approach is thermal analysis (TA). It may provide information on change of state (e.g. polymorphism, evaporation, transition and melting temperature) and purity of the drug substance. Techniques most often used in TA are differential scanning calorimetry (DSC) and thermogravimetric analysis (TG).

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To characterize the drug substance, a preliminary examination over a wide range at a high heating rate (10°C per min) is performed. Transitions of interest are then examined at a lower heating rate (about 1°C per min).

Impurity analysis

A rise in the impurity level influences the DSC melting peak shape by lowering the melting point and broadening the melting range. This effect is illustrated in Fig. 1 for samples of penfluridol differing by a few tenths percent in impurity content.

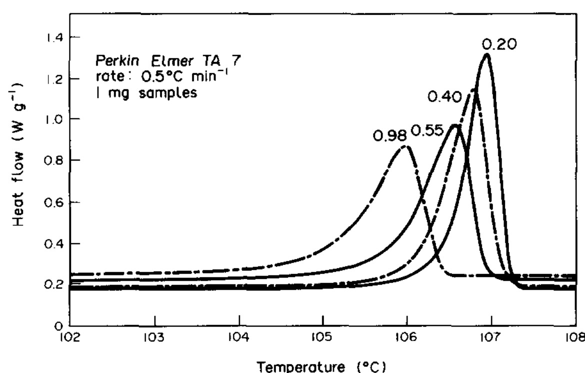

Figure 1
Influence of impurity level (in mole %) on DSC melting peak shape for penfluridol.

The relationship between molar fraction of impurity and melting-point depression is given by the equation:

$$X_2 = \frac{(T_0 - T_m)\,\Delta H}{R T_0^2},$$

where T_0 = melting point of the pure compound (K)
T_m = melting point of the test specimen (K)
ΔH = molar heat of fusion of the major component (Joule mole^{-1})
R = gas constant (8,314 Joule.K^{-1}.mole^{-1})
and X_2 = molar fraction of minor component (= impurity).

A plot of the observed test specimen temperature versus the reciprocal of the melted fraction should yield a straight line with the slope equal to the melting point depression [1]. For attainment of this linear plot, the impurity must be soluble in the liquid phase or melt but insoluble in the solid phase. Impurities forming solid solutions are not detected by DSC so that purity results are too high [2]. For ideal compounds, DSC-results are reproducible within 0.1%. A typical thermogram for penfluridol is given in Fig. 2.

Limitations for DSC are solid solution formation, insolubility in the melt and decomposition during the analysis.

Polymorphism

Many compounds are capable of crystallizing in more than one type of crystal form, i.e. they display polymorphism. At any particular temperature and pressure, only one polymorph is thermodynamically stable. Since the rate of phase transformation of one

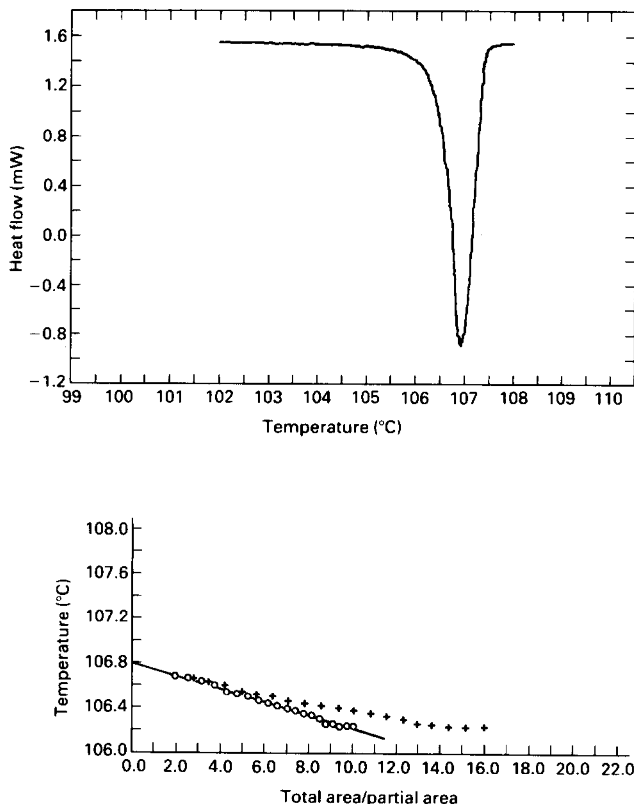

Figure 2
(a) Purity determination by differential scanning calorimetry for penfluridol: DuPont model 1090; initial temperature, 102°C; heating rate, 0.5°C min^{-1}; final temperature, 108°C; nitrogen flow rate, 50 ml min^{-1}; sample weight, 2.4 mg; sample holder, aluminium. (b) Purity determination by DSC for penfluridol: depression of melting-point: purity, 99.79 mole %; ΔH, 41.5 kJ mol^{-1}; m.p., 106.8°C; m.p. depression, 0.06°C; correction, 4.13%; MW, 524.0; cell constant, 0.932; initial slope, −14.55 mW/°C. Experimental values, +; corrected values, O.

polymorph to another can be slow, it is possible to find several polymorphs of a drug substance existing under normal handling conditions. Just as every polymorph has its own characteristic IR absorption spectrum and X-ray diffraction pattern, DSC is useful for detecting and therefore for monitoring polymorphism.

An example of DSC detection of polymorphism for terconazole is given in Fig. 3.

Solvent loss

Thermogravimetry is a technique in which the mass of a substance is monitored as a function of temperature as the sample is subjected to a controlled temperature program. In TG only those changes in properties associated with a change in mass are observed [3].

Loss of water from the crystal lattice in a hydrate can be distinguished from loss of adsorbed water. This is illustrated in Fig. 4 for anhydrous oxatomide, oxatomide monohydrate and oxatomide with a small amount of adsorbed water.

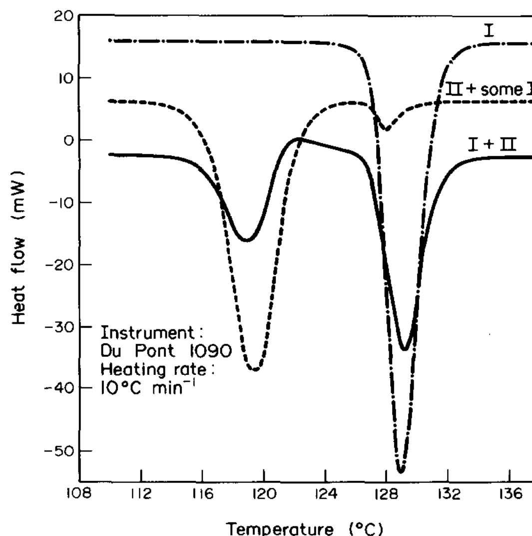

Figure 3
Detection of polymorphism by DSC for terconazole.

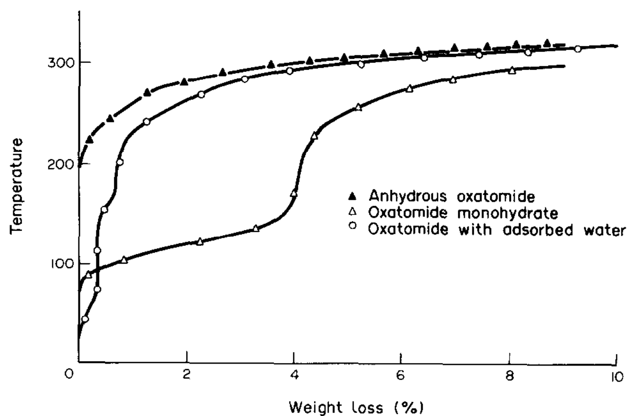

Figure 4
Thermogravimetry of oxatomide samples.

Chromatographic Approach to Purity Determination

Purity values of drug substances obtained by different analytical methods are expressed in different units and can be inter-related only when the system is fully defined, which is rare.

In order to give some meaning to the estimate of purity, batches of the synthesized drug substance are examined under several chromatographic conditions and methods. Structural information on impurities is achieved by chromatography coupled to mass spectrometry (GC–MS or HPLC–MS). In daily practice these coupling devices offer

unique possibilities for identification and have a tremendous impact in the analysis of very small amounts of components in complex mixtures [4]. Batches may also be recrystallized to concentrate the impurities in the mother liquors and then be subjected to preparative chromatography. The structures of the isolated impurities are then deduced from spectral examination (UV, IR, NMR and MS). In both approaches, confirmation is obtained by independent synthesis.

The purity estimate refers only to a drug substance synthesized by one specific route. Any change of route may lead to a different impurity profile and must therefore be similarly investigated. The best technique for purity control of the test material is to determine the individual impurities by evaluation against suitable standard solutions of the individual impurities. This technique, however, does require a supply of standard impurities of defined purity. Each development analyst is responsible for finding the most suitable method for the analysis of a well-defined drug substance.

Gas chromatography

The major limitation of GC is that samples or their derivatives must be volatile and thermostable. The most important recent development in GC has been the introduction of fused silica capillary columns. Because of the high resolution and short analysis times possible with open tubular columns, this technique has become popular [5]. GC is very well suited for quantitative analysis. For structural studies GC is commonly coupled to a mass spectrometer.

Liquid chromatography

The major advantage of HPLC is that it is a milder method than GC since it operates at ambient temperature. HPLC is suitable for the more polar, less volatile samples that are not amenable to analysis by GC and demonstrate a very high separation power. The introduction of the diode array enables simultaneous measurements to be made at different UV-wavelengths. Despite its high equipment cost, HPLC is often a slow technique even with the use of an autosampler.

The development of a suitable interface for LC–MS has been slow [6]. Two important types of interface system exist. The first, called 'moving belt' is a transport system that carries solute and solvent on a moving band to an oven where the solvent is removed by evaporation; the residual solute is passed directly into the MS source where it is thermally vaporized. The electron impact ionization spectra that are also obtained are useful for structure elucidation. The second type of interface is based on a thermospray technique. Thermospray ionization is effected by means of an electrolyte in the liquid stream and the buffer most commonly added is ammonium acetate [7].

Thin-layer chromatography

TLC is probably the most extensively used method. To visualize the spots on the TLC-plate staining with iodine, a UV light source or different specific colour reagents may be used. It offers the advantage of an open bed chromatography. Quantification may be achieved by TLC scanners, but it remains difficult for components that are eluted close to the main compound.

The economic aspect of the selection of a certain chromatographic technique for obtaining the purity estimate is a major factor [8]. Some of the factors contributing to the economic acceptability of different chromatographic techniques are summarized in Table 1.

Table 1
Economic acceptability of different chromatographic techniques

Technique	Equipment costs	Maintenance costs	Operator costs
TLC			
visual	low	low	low
instrumental	high	moderate	low
GC			
without autosampler	high	moderate	moderate
with autosampler	very high	moderate	low
HPLC			
without autosampler	very high	moderate	moderate
with autosampler	very high	moderate	low

Nature and Significance of Impurities

An impurity in a drug substance means any substance other than the active compound and applies to various components, e.g. remaining intermediates, residual solvents, side-reaction products, decomposition products, isomers and polymorphs. This may be illustrated by some examples.

Impurity profile of haloperidol decanoate

Haloperidol decanoate is a long-acting neuroleptic compound. Its therapeutic effect is based on the slow release of haloperidol from its decanoate ester by enzymatic hydrolysis so that a stable effect is obtained for at least one month [9].

Two major impurities are found in the drug substance: haloperidol and haloperidol dodecanoate. During synthesis of the decanoate ester starting from haloperidol (Fig. 5), hydrolysis of the decanoate ester also takes place; thus haloperidol can be regarded as a decomposition product and will be present as an impurity in the drug substance. Haloperidol dodecanoate is found as an impurity since decanoyl chloride always contains dodecanoyl chloride as an impurity. Other minor impurities that are found have their origin in impurities present in haloperidol or in the remaining decanoic acid.

The role of isomerism

Of the various types of stereochemistry, the occurrence of enantiomers is of particular significance. If the centre of asymmetry is located in a section of the molecule that participates in the drug-receptor interaction, it influences the activity of the compound.

Stereoisomers are different chemicals, mostly with quite distinct biological properties. The majority of drug substances contain asymmetric centres and, hence, they can exist in two isomers. Often only one isomer is therapeutically active. The therapeutically non-active isomer in a racemate should be regarded as an impurity; it may contribute to side-effects and risks associated with the drug and, whenever possible, its presence should be avoided [10].

The catastrophe of thalidomide is one well known example where the teratogenic effect is caused by the (−)-(S)-form whereas the (+)-(R)-form is responsible for the sleep-induced effect [11]. The development of stereospecific syntheses and methods for the separation of optical isomers is a challenge for medicinal chemists and opens

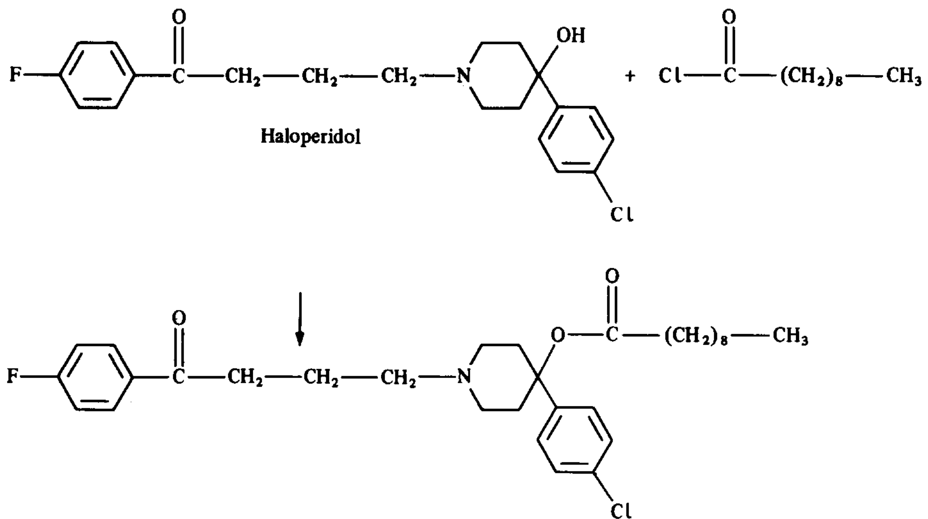

Figure 5
Synthesis of haloperidol decanoate.

perspectives for more selective and safer therapeutics. Particularly interesting is the example of the new antihistaminic compound levocabastine [12]. From the four racemates of 1-[4-cyano-4-(4-fluorophenyl)cyclohexyl]-3-methyl-4-phenyl-4-piperidine-carboxylic acid, cabastine was found to be the one with the most potent antihistaminic action.

After resolution of cabastine into its two optical isomers, levocabastine was found to be about 90 times more potent and 5 times safer than dextrocabastine. For those reasons the higher cost of synthesis of levocabastine compared with that of cabastine is justified. Especially where differences in activity may be encountered, synthesis of only one of the optical isomers in the absence of the other is necessary, even if this process is difficult and costly.

Comments on Limits of Impurities

Limits are presented in different ways: a limit for the total amount of all possible impurities; and limits for individual impurities based upon toxicological properties and actual levels of impurities in batches already manufactured. Impurities should be kept at as low a level as is reasonably possible and should exceed 0.5% only when purification cannot economically reduce them further. The impurity profile of a drug substance is characterized by the method of synthesis of the compound. Methods of synthesis and, hence, impurity profiles can vary greatly among different manufacturers.

Identification, with proof of structure by independent synthesis if feasible, is generally needed for impurities present in proportions of 0.1% or above. If the structure is similar to a known carcinogen, mutagen or other hazardous compound, toxicological and pharmacological data must be obtained.

To validate chromatographic methods, it must be demonstrated that each impurity is resolved from the active ingredient. The method should be sufficiently sensitive to detect impurities at levels below their permissible limits.

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