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Review

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

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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

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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

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essential factor in ensuring drug quality, regulatory agencies seem to have been much less rigorous and demanding in the past, due to the lack of appropriate analytical means. Release of, for example, a batch of drug substance which could be done within a day several decades ago, will now require a week. 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 least, benzene is a potential contaminant that has attracted media attention.

Maintaining a consistent impurity profile for an active ingredient according to the ICH guidelines is no easy task for the pharmaceuticals manufacturer and for suppliers of raw materials and intermediates; 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

polymorphism or pseudo-polymorphism (solvation). Thus identification also helps to characterize the solid state.

2.2. Characterising the solid state

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 well characterised compounds, despite the fact that polymorphs rarely give completely different spectral bands, their presence manifesting itself in different band ratios. Sometimes, grinding with KBr, compression to form a pellet, and even the heat from the IR light source can provide sufficient energy to cause 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

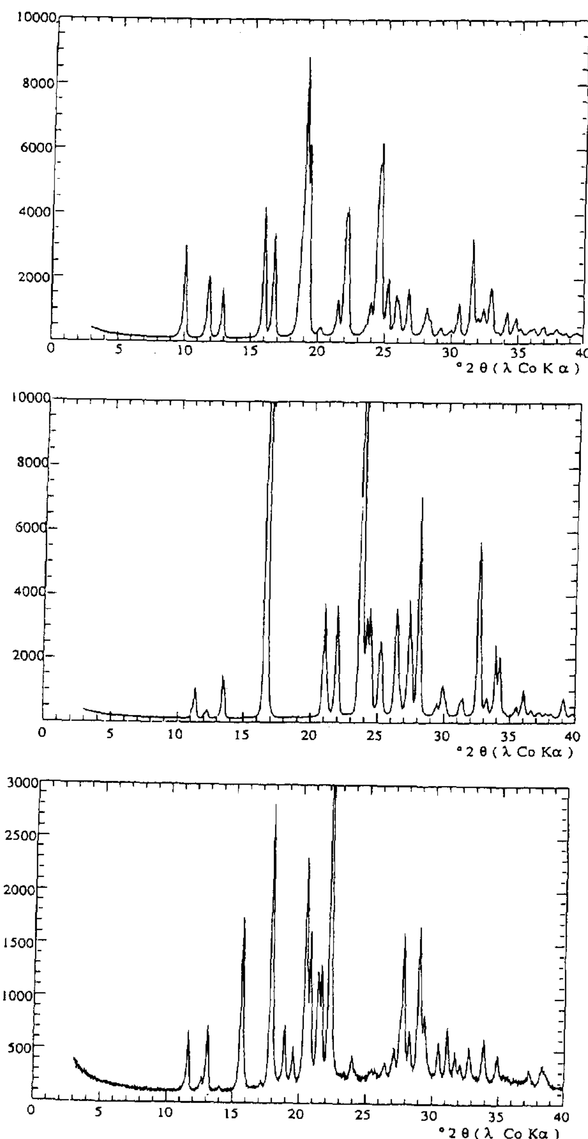

Fig. 1. X-ray powder diffraction patterns of 3 polymorphs of a drug substance. Apparatus: Philips PW 1710; X-ray tube: λCoKα, 40 kV, 40 mA.

(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

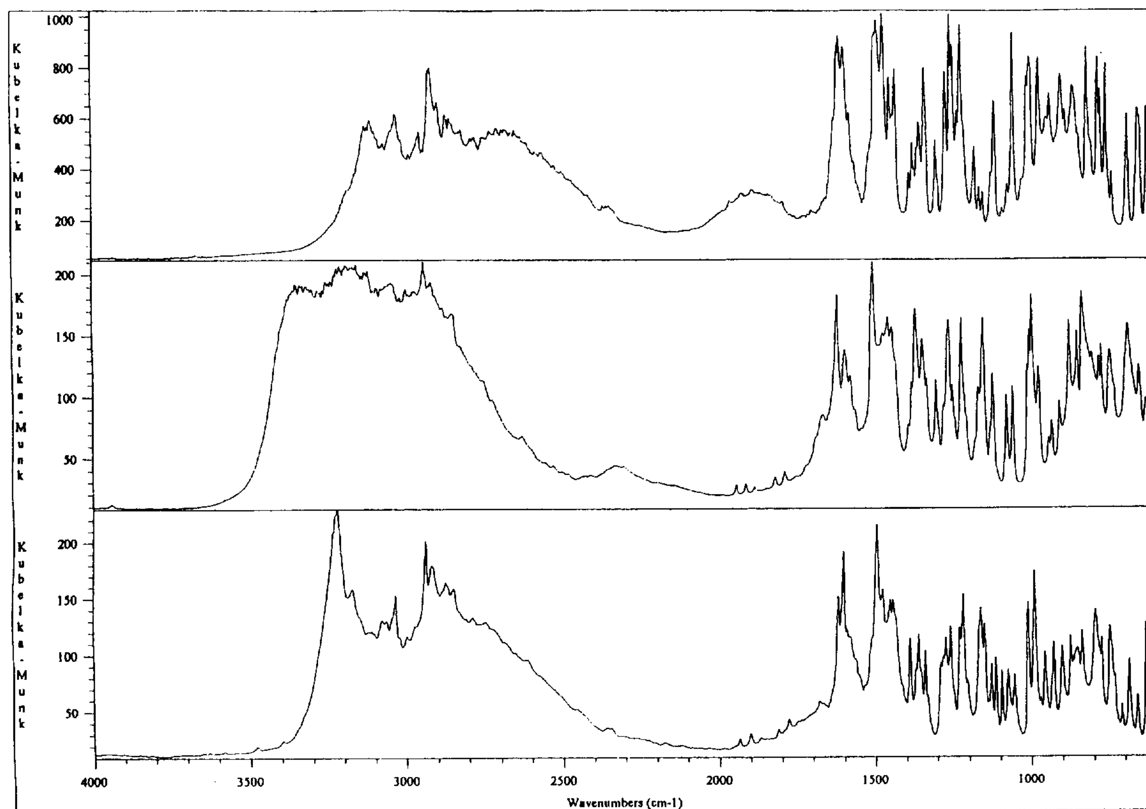

Fig. 2. Diffuse reflection IR spectra of 3 polymorphs of a drug substance. Apparatus: Nicolet, Magna-IR 500 Spectrophotometer with Spectra-Tech diffuse reflection accessory.

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-

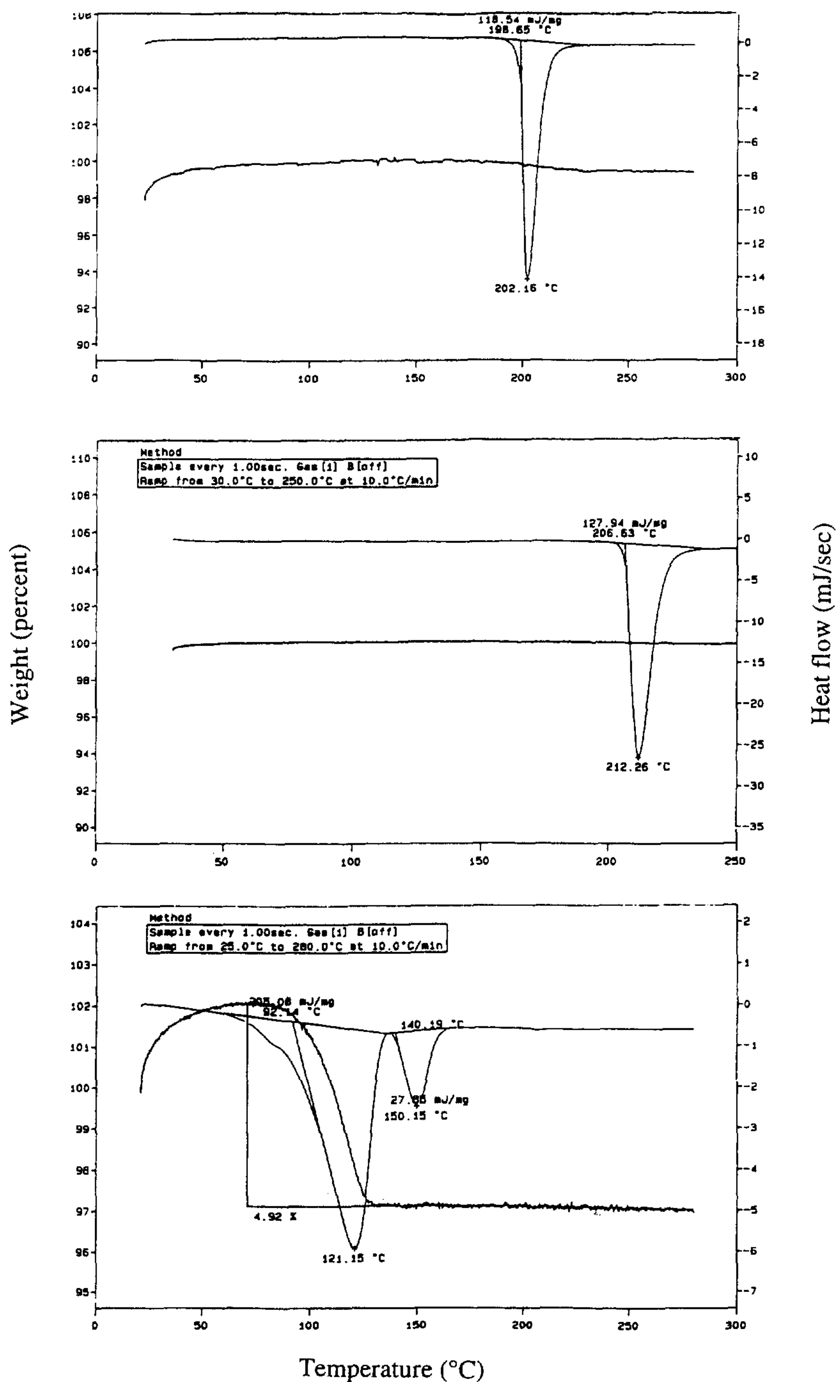

Fig. 3. DSC-TGA curves of 3 polymorphs of a drug substance. Apparatus: Polymer Laboratories STA 625.

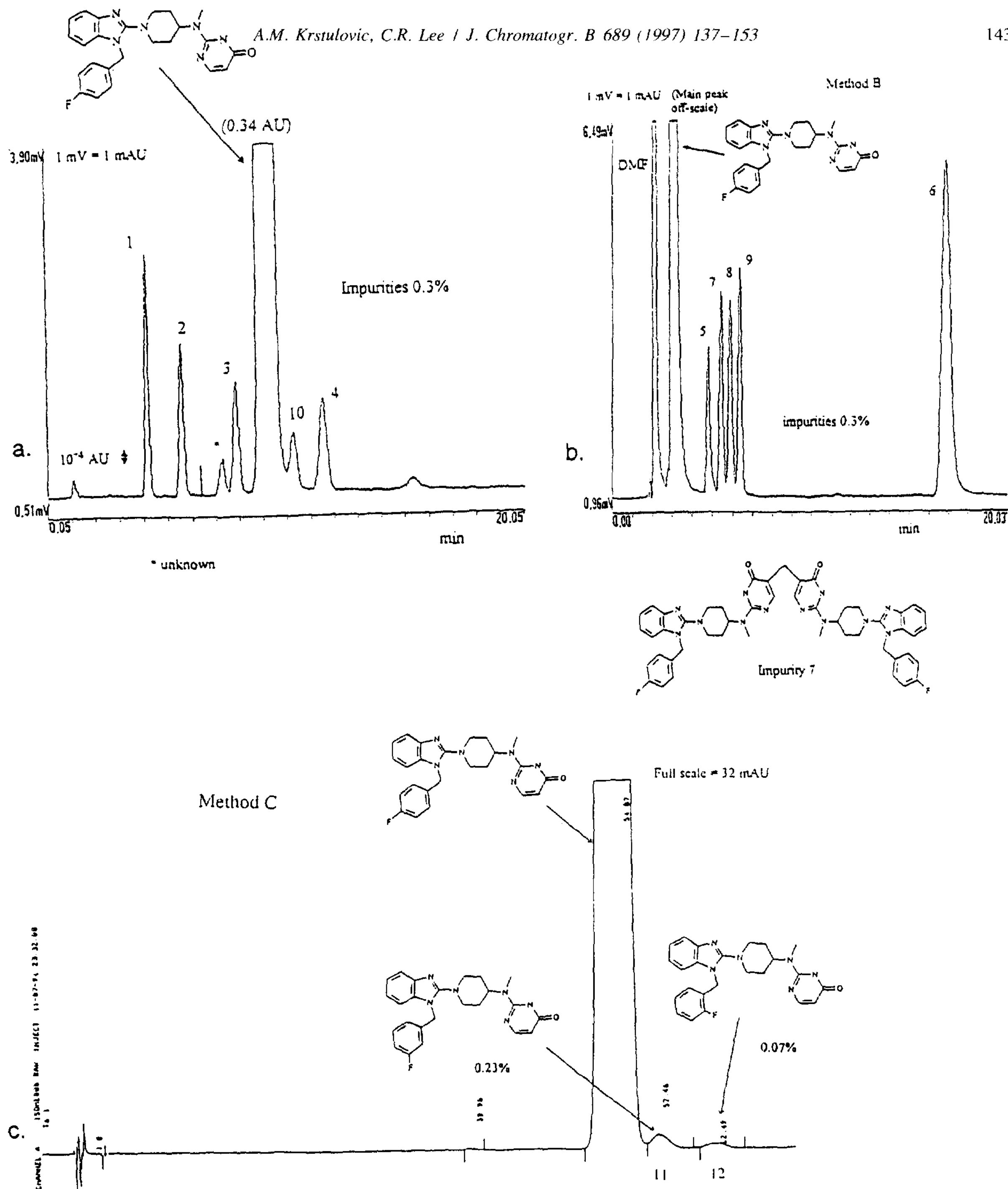

Fig. 4. HPLC analysis of 11 potential impurities of mizolastine. Chromatographic conditions: (a) Column: Inertsil ODS (150×4.6 mm I.D., 5 μm), G.L. Sciences; mobile phase: 55:15:30 (v/v) mixture of 0.05 *M* KH_2PO_4 (pH 3.5), acetonitrile and methanol; flow-rate: 1.5 ml/min; detection: UV absorbance at 254 nm. (b) Column: Inertsil C_8 (150×4.6 mm I.D., 5 μm); mobile phase: 35:30:35 (v/v) mixture of 0.05 *M* KH_2PO_4 (pH 3.5), acetonitrile and methanol; flow-rate: 1.5 ml/min; detection: UV absorbance at 254 nm. The first peak denoted by DMF is dimethylformamide used for the preparation of the reference solution of impurities. (c) HPLC separation of the two positional isomers of mizolastine. Column: Inertsil C_8 (150×4.6 mm I.D., 5 μm); mobile phase: 60:40 (v/v) mixture of 0.05 *M* KH_2PO_4 (pH 3.5), containing 0.01% of N,N-dimethyloctylamine and methanol; flow-rate: 0.9 ml/min; detection: UV absorbance at 280 nm.

ceutical analysis. It is, however, essential for the quality control of solvents and aromas, for the determination of residual solvents and, (in a few cases where other methods fail) for the determination of isomeric impurities. TLC, which had been relegated to a complementary role, is now a serious rival to HPLC, through the development of automatic equipment that includes a sensitive and accurate scanner. The capital cost per instrument set is relatively high, but this is more than offset by fast sample throughput and low solvent costs. Capillary electrophoresis (CE) is gaining widespread popularity as a separation tool because of its high separating power, low running costs and negligible solvent consumption. In contrast to HPLC where column selectivity, a key factor in obtaining a separation, is usually a trade secret, the separation conditions in CE can be fully defined, at least in principle. Although the number of applications of CE in pharmaceutical research and quality control is impressive and growing every day, this technique (coupled with UV absorbance detection) still suffers from relatively low sensitivity, modest repeatability and variations in the physico-chemical state of the column wall. In particular, detection limits for impurities are not often much better than 0.1% with respect to the main peak, a level that would only just be acceptable as a quantification limit. An example reflecting the current state of the art of a CE separation of related substances of fluparoxam is shown in Fig. 5.

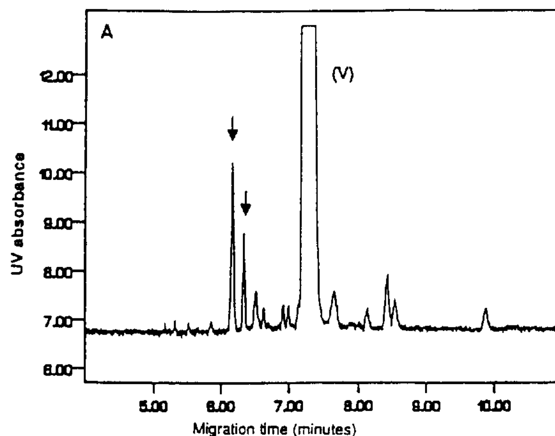

Fig. 5. CE separation of fluparoxan drug substance. Separation conditions: 50 m*M* sodium tetraborate adjusted to pH 2.2 with conc. H_3PO_4; sample concentration: 0.5 mg/ml in water; column length and diameter: not stated; detection: 214 nm; 10 s pressure injection; 10 kV. Reproduced from Ref. [3] with permission.

Chiral compounds may contain enantiomeric impurities which, in the opinion of these authors, should be treated as any other organic impurities unless they are particularly toxic. With the major advances that have been made in the development of chiral stationary phases, detection of chiral impurities either by HPLC or by supercritical fluid chromatography (SFC) has become routine. For a given column, SFC generally gives better separations than HPLC, largely because of better chromatographic efficiency; separations are also faster. Relatively low running costs (CO_2 is ten times cheaper than acetonitrile) tend to offset the higher cost of the equipment. One disadvantage is a relatively noisy baseline with UV absorbance detection. Fig. 6 shows an SFC separation of a racemic mixture of eliprodil hydrochloride: because of the high efficiency of the separation, trace amounts of enantiomers can be detected. Nevertheless, because separations are performed in non-aqueous media (normal-phase), SFC is less useful for pharmacokinetic studies, nor does it seem likely to become the dominant separation technique in quality control. Chiral impurities can also be conveniently searched for using CE with chiral buffer additives (e.g., cyclodextrins).

Inorganic impurities present in pharmaceutical products originate from the equipment used and from reagents, catalysts, drying agents and filter aids. They are classically determined using pharmacopoeial methods, such as the heavy metals test (precipitation of sulphides) and sulphated ash, the usual specifications being 10–20 ppm and 0.1%, respectively. Needless to say, any particularly toxic elements that might be present should be assayed specifically and the corresponding acceptance limits established. Atomic absorption spectroscopy is commonly used; although only one or a few elements can be determined at a time, the equipment is relatively inexpensive and the use of electrothermal atomisation generally eliminates the need for wet washing. Both the global and element-by-element approaches might eventually be replaced by specific multielemental analysis techniques.

Solvents, which may be inorganic or organic, are important in the chemical synthesis of active ingredi-

ELIPRODIL: CHIRAL SEPARATION

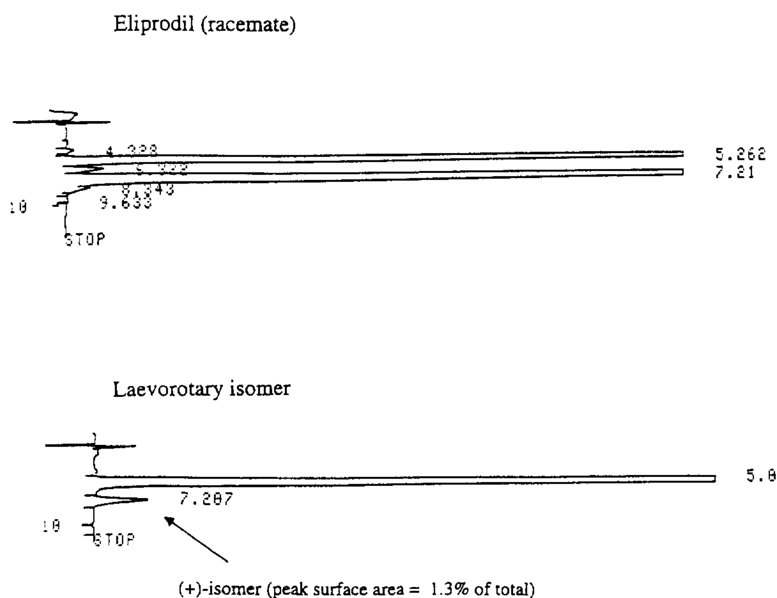

Fig. 6. SFC separation of enantiomers of eliprodil (top) racemic mixture, (bottom) laevorotatory isomer spiked with 1.3% of the dextrarotatory isomer. Chromatographic conditions: column: Chiralpak AD (250×4.6 mm I.D., 10 μm) (DAICEL); mobile phase: 80:20% mixture of CO_2 and methanol containing 0.2% diethylamine; pressure: 20 mPa; temperature: 22°C; flow-rate: 3.0 ml/min; detection: UV absorbance at 225 nm.

ents since, when properly chosen, they can enhance the yield and influence crystalline form, purity and solubility. They are also used for wet granulation in the manufacture of drug products. Residual solvents and other volatile impurities must be detected and assayed, not only because of their potential toxicity and deleterious environmental effects, but also because they can impart undesirable organoleptic characteristics to drugs. Current ICH guidelines classify organic solvents into three classes: class 1 comprises known human carcinogens, strongly suspected carcinogens or environmental hazards: benzene (2 ppm), 1,1,1-trichloroethane (1500 ppm), 1,2-dichloroethane (15 ppm) and carbon tetrachloride (4 ppm). These solvents should not be used in the production of pharmaceutical products. The second class of organic solvents comprises 22 non-mutagenic animal carcinogens, and neurotoxic and teratogenic substances: dichloromethane, 1,4-dioxane, etc. Their limits can be expressed either in ppm or in terms of 'permitted daily exposure' (PDE) if the daily dose is known and fixed. Class 3 contains 35 solvents of low toxic potential to humans and the environment: acetone, ethyl acetate, 2-propanol, ethanol, etc., for which there is no need to set **health-based** exposure limits. Amounts of up to 0.5% can be accepted without justification. Residual solvents are assayed by capillary GC with flame ionisation detection: the preferred method is static headspace analysis, but in special cases direct injection and purge and trap (dynamic headspace) systems can be used. Identification can conveniently be carried out by GC–MS, although because of the high resolution and reproducibility of retention times afforded by contemporary GC capillary columns, safe attribution of peaks can be achieved by the use of two columns in parallel. Fig. 7 shows the retention properties of selected solvents on silicone (OV1701 equivalent) and polyethyleneglycol stationary phases. An example of the application of static

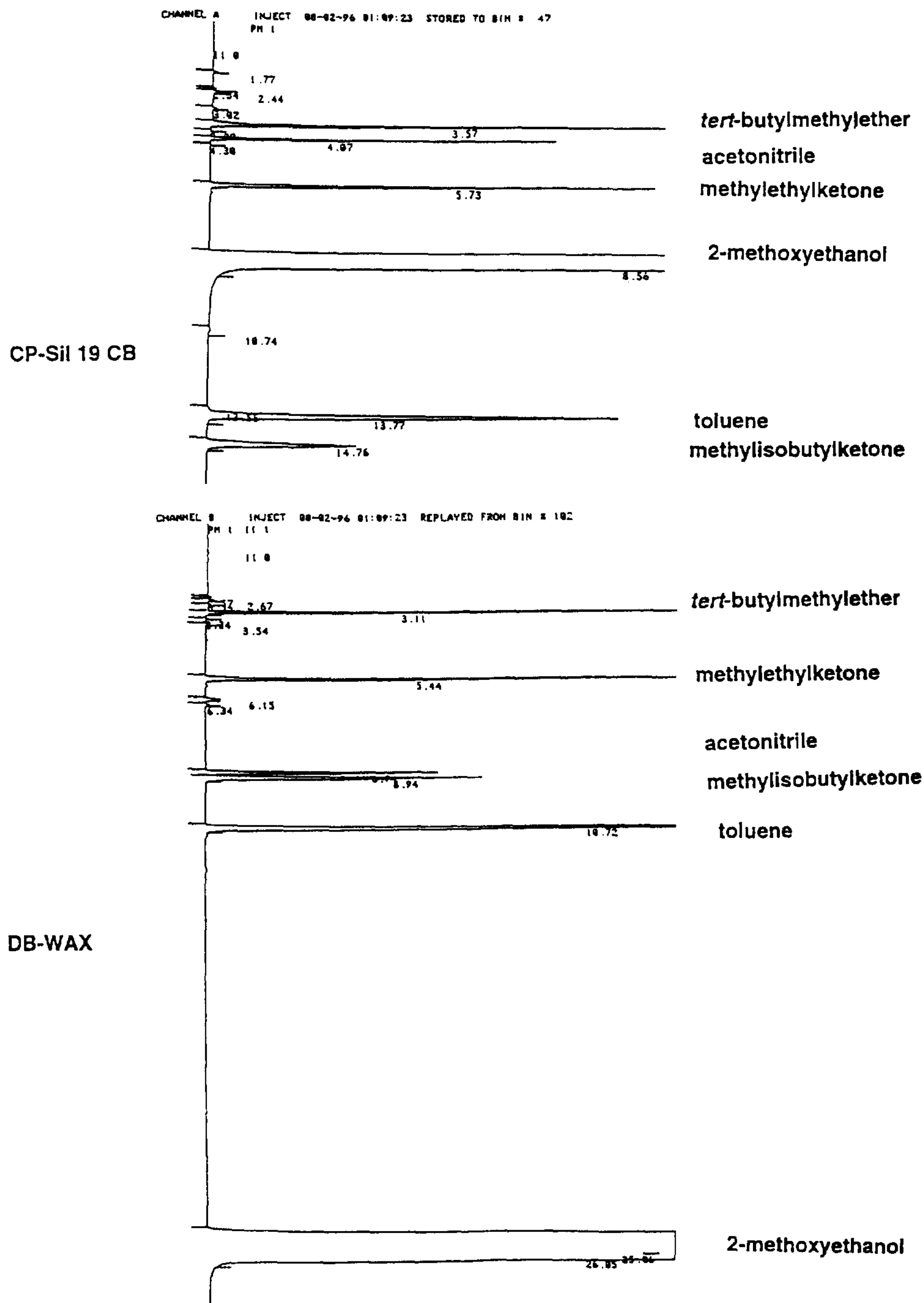

Fig. 7. Dual-column capillary GC head-space analysis of selected residual solvents with 2-methoxyethanol as dissolution solvent. Chromatographic conditions: (top) column 1: CP-Sil 19 CB (cyanopropylphenyldimethylsilicone) (50 m×0.32 mm, d_f=1.2 μm); (bottom) column 2: DB-WAX [poly(ethylene glycol)] (60 m×0.32 mm, d_f=0.5 μm); apparatus: Perkin-Elmer HS40 headspace sampler connected to Auto System GC through split injector. The columns are connected to the injector by means of a 2-hole ferrule. Carrier gas (He): 30 p.s.i. at autosampler, 25 p.s.i. at injector, split flow-rate: 60 ml/min; detector: FID; temperatures: columns: 50°C; injector: 75°C; detectors: 300°C; head-space sampler: 65°C.

headspace GC analysis of residual solvents of eliprodil hydrochloride is shown in Fig. 8. Care must be taken in interpreting the results as numerous volatile impurities other than the solvents themselves are detected: relatively involatile solvent impurities may become concentrated, many solvents contain stabilisers, some grades of alcohol are denatured. In addition, various reactions can take place either during recrystallisation and drying or in the sample vial: for example, acetone can give rise to mesityl oxide and mesitylene, and ethanol to diethyl ether.

2.4. Limits for impurities; qualification

Acceptable levels for organic impurities and solvents depend on the following factors: toxicity, feasibility and cost of synthesis, analytical feasibility, as well as the route of administration (oral intake vs. i.v.) of the corresponding drug products, duration of treatment and the dose administered. ICH guidelines (Table 1) require that limits be set for impurities in drug substances above the arbitrary 0.1%, although lower limits should be set for particularly toxic

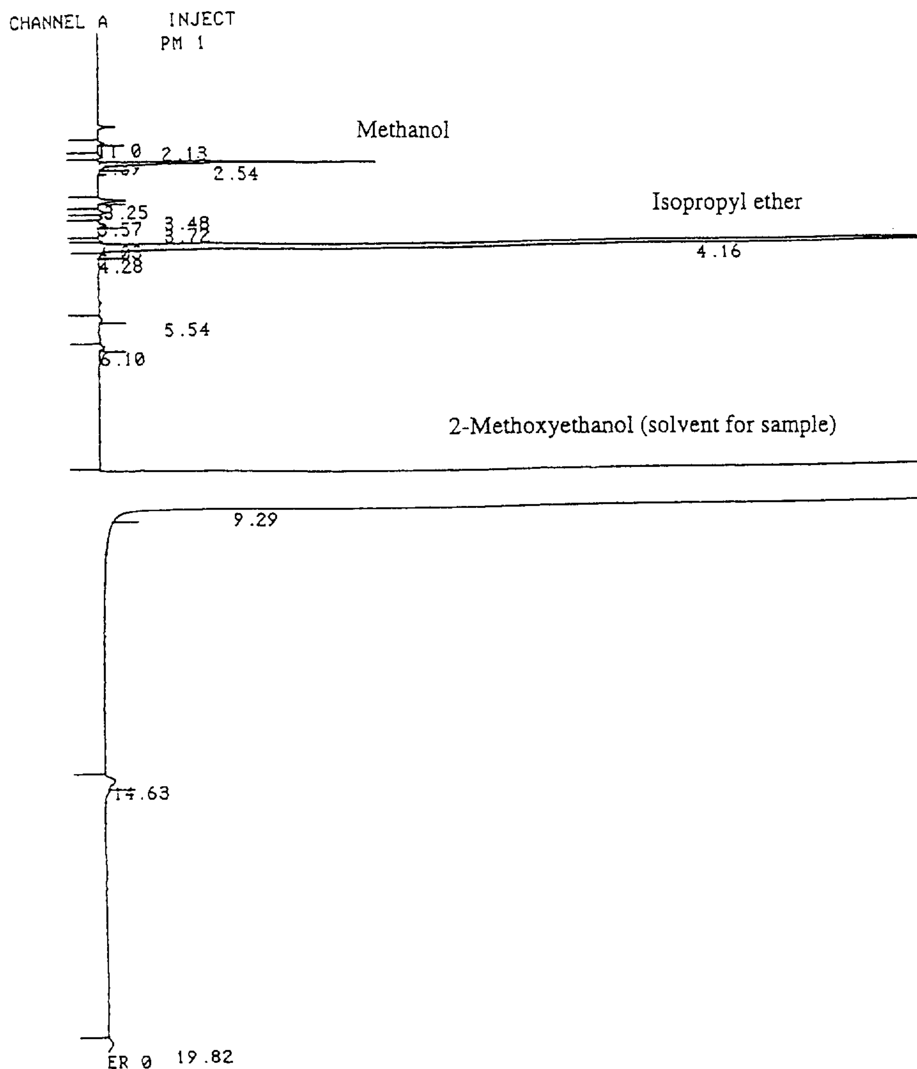

Fig. 8. Capillary GC head-space analysis of residual solvents eliprodil hydrochloride. Solvent: 2-methoxyethanol. The chromatographic conditions are the same as in Fig. 7(top); chromatographic column 1.

Table 1

Maximum daily dose	Qualification threshold
≤2 g/day	0.1% or 1 mg per day intake (whichever is lower)
>2 g/day	0.05%

impurities (e.g., 1 ppm for ethylene oxide). Above this level all unknown impurities should be identified. More importantly, all impurities above 0.1% should be qualified by appropriate toxicological studies. In order to facilitate drug development, early batches of drug substances should contain all impurities susceptible to be present in clinical batches. Thus the quality of the batches used in clinical trials should be at least equal to and, if possible, higher than that of the early batches used in preclinical tests.

2.5. Assay

Drug substances are assayed chromatographically and (if a titratable function is present) titrimetrically. The latter method is less specific, but it does not depend on the purity (or existence) of a reference substance and it is often more accurate and precise, the relative standard deviation generally being <0.3% for a potentiometric assay. This degree of precision does not, however, allow any comparison to be made between the assay and the impurity content.

3. Drug products

Purity requirements for drug products are more difficult to establish than those for the drug substances, since factors such as maximum daily dose, duration of treatment, route of administration, etc., have to be taken into account.

In the case of drug products containing drug substances which exhibit polymorphism, care must be taken to ensure that manufacturing leads consistently to the same polymorph. This is most commonly verified by dissolution testing.

3.1. Impurities

Impurities contained in new drug products comprise impurities and degradation products of the synthetic ingredient, products arising from reactions between the active ingredient and excipients, manufacturing equipment or the immediate container/closure system, contaminants (GMP issue) and solvents used in the manufacturing process.

Residual solvents in pharmaceutical preparations resulting from manufacturing are of lesser concern, because the choice is usually limited to water and ethanol. However, drug products should be tested if Class 1 or Class 2 solvents are used for manufacturing.

3.2. Limits for impurities

Acceptance limits proposed by ICH (step 2) for the organic impurities present in drug products are considerably higher than those for drug substances [2]. This reflects the fact that pharmaceutical preparations are usually less stable than pure drug substances. Table 2 gives thresholds for identification and qualification of impurities in new drug products. It is important to point out that additional safety testing is required for impurities above the threshold level not already present in drug substances and thus not covered by toxicological studies.

Contamination of pharmaceutical preparations during manufacture is a subject of particular interest. When this is detected it is mandatory to unambiguously identify the contaminant prior to reaching any decision concerning the acceptance of the product, regardless of its level. This point is illustrated in Fig. 9a which shows the HPLC profile of a sorbitol-containing placebo solution for parenteral use in which extraneous peaks were detected under the protocol analytical conditions. HPLC analysis of a

Table 2
Thresholds for identification and qualification of degradation products in new drug products

Maximum daily dose[a]	Threshold
Identification	
<1 mg	1.0% or 5 μg TDI[b] whichever is lower
1 mg–10 mg	0.5% or 20 μg TDI whichever is lower
>10 mg–2 g	0.2% or 2 mg TDI whichever is lower
>2 g	0.1%
Qualification	
<10 mg	1.0% or 50 μg TDI whichever is lower
10 mg–100 mg	0.5% or 200 μg TDI whichever is lower
>100 mg–2 g	0.2% or 2 mg TDI whichever is lower
>2 g	0.1%

[a] The amount of drug substance administered per day.
[b] Total daily intake.

hexane extract with detection at 230 nm showed the presence of 2 extraneous peaks (labelled A and B), confirmed by the LC-particle-beam–MS total ion current chromatogram. They were identified as *n*-butylbenzenesulfonamide and dehydroabietic acid and originate probably from the plastic materials used in manufacturing (Fig. 9b).

Leaching from container/closure systems can also be a source of impurities. Their importance depends on many factors. For example, when chlorobutyl rubber stoppers partially coated with teflon in order to reduce leaching while retaining good sealing properties were left in contact for 1 month with a 1:1 ethanol–water mixture at 65°C, the chromatogram of the extract showed, not unexpectedly, the presence of sulphur, dibutylphtalate and butylhydroxytoluene, in addition to other trace compounds. The extent of migration of plastic additives can depend inter alia on the shape of the bottle neck (Fig. 10), which determines the area of contact between the solution and the non-coated section of the stopper. The choice of detection system is of course critical when assessing the acceptability of analytical results. Fig. 11 shows the profiles of the leachable substances in the same sample detected by UV absorbance at 240 nm, evaporative light scattering and mass spectrometry with a particle beam interface. This figure clearly demonstrates the importance of the choice of appropriate method for reaching correct conclusions.

4. Conclusion

In conclusion, the assessment of drug purity in accordance with present standards requires the use of state-of-the art validated analytical methods which often involve relatively sophisticated techniques. Analysts and toxicologists, who should work hand in hand, need to decide what levels of impurities should be detected for safety reasons or in order to be sure that the manufacturing process is under control. While the use of increasingly sensitive detection devices reinforces the need for critical judgement in interpreting results, there appears to be a satisfactory match, in the case of drug substances, between the norms that have been agreed upon and the range and performance of the analytical methods currently in use.

Finished drug products present a more difficult problem because there is a wider range of possible degradation products which may be present at levels exceeding those authorized by toxicological studies of the drug substance, and a greater risk of contamination through interactions with container/closure systems. Generally speaking, release of drug products relies at present too heavily on the release of the drug substance. A frequently asked question that has no objectively justifiable answer concerns the degree of analytical eagerness that one must exercise before concluding that a product is sufficiently pure. No sufficiently sensitive LC or CE detector exists that gives a constant and linear mass-sensitive response for every analyte. Establishing norms for contaminants is another major issue; whereas traces of compounds such as *p*-hydroxybenzoic and phthalic esters that commonly find their way into finished products should not cause too much concern provided their source can be traced, it must not be assumed that all plastics additives (for example) are harmless.

Finally, carefully conducted stability studies under controlled conditions of heat, humidity and exposure to light must provide reliable information for establishing end-of-shelf-life specifications for all climatic zones in which the drug product is to be marketed. While ICH guidelines for proper evaluation of light sensitivity are under way, it seems regrettable that the conditions of temperature and humidity used for

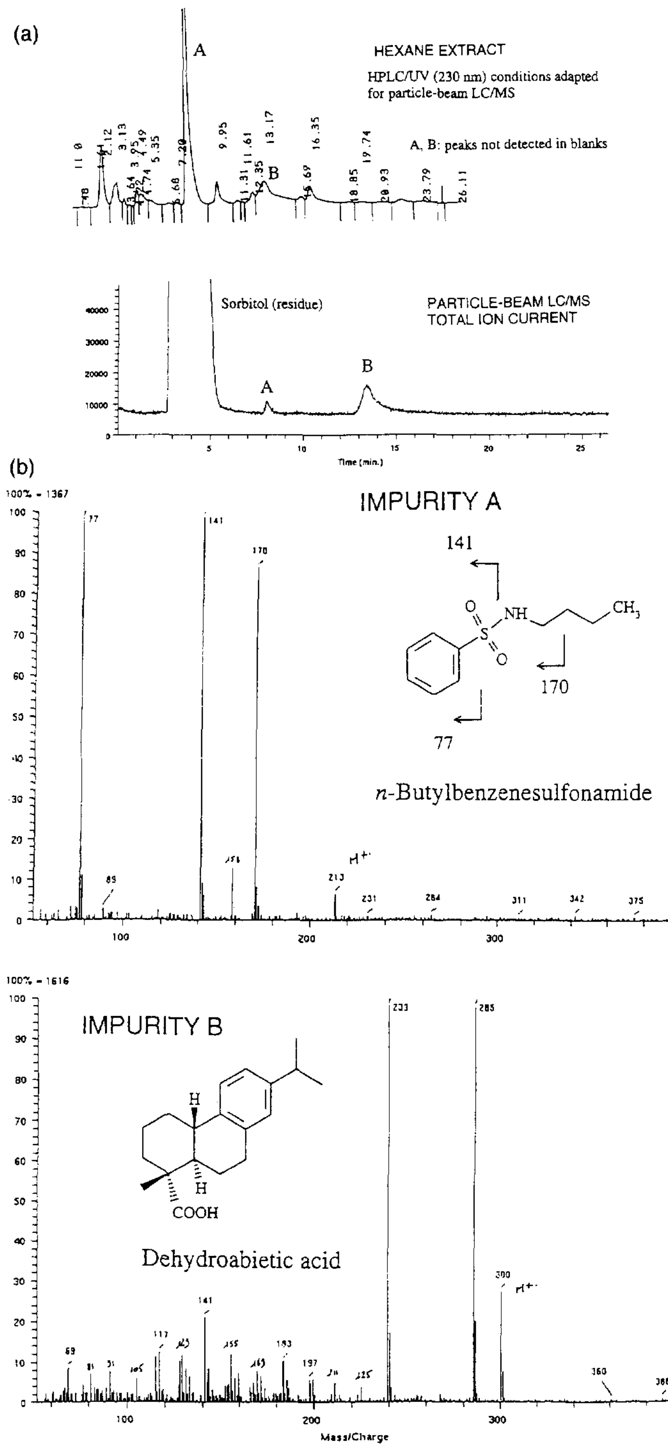

Fig. 9. (a) HPLC profile and the particle beam/LC–MS total ion current of a hexane extract of a placebo solution for parenteral use. (b) Mass spectra of the 2 impurities labelled A and B.

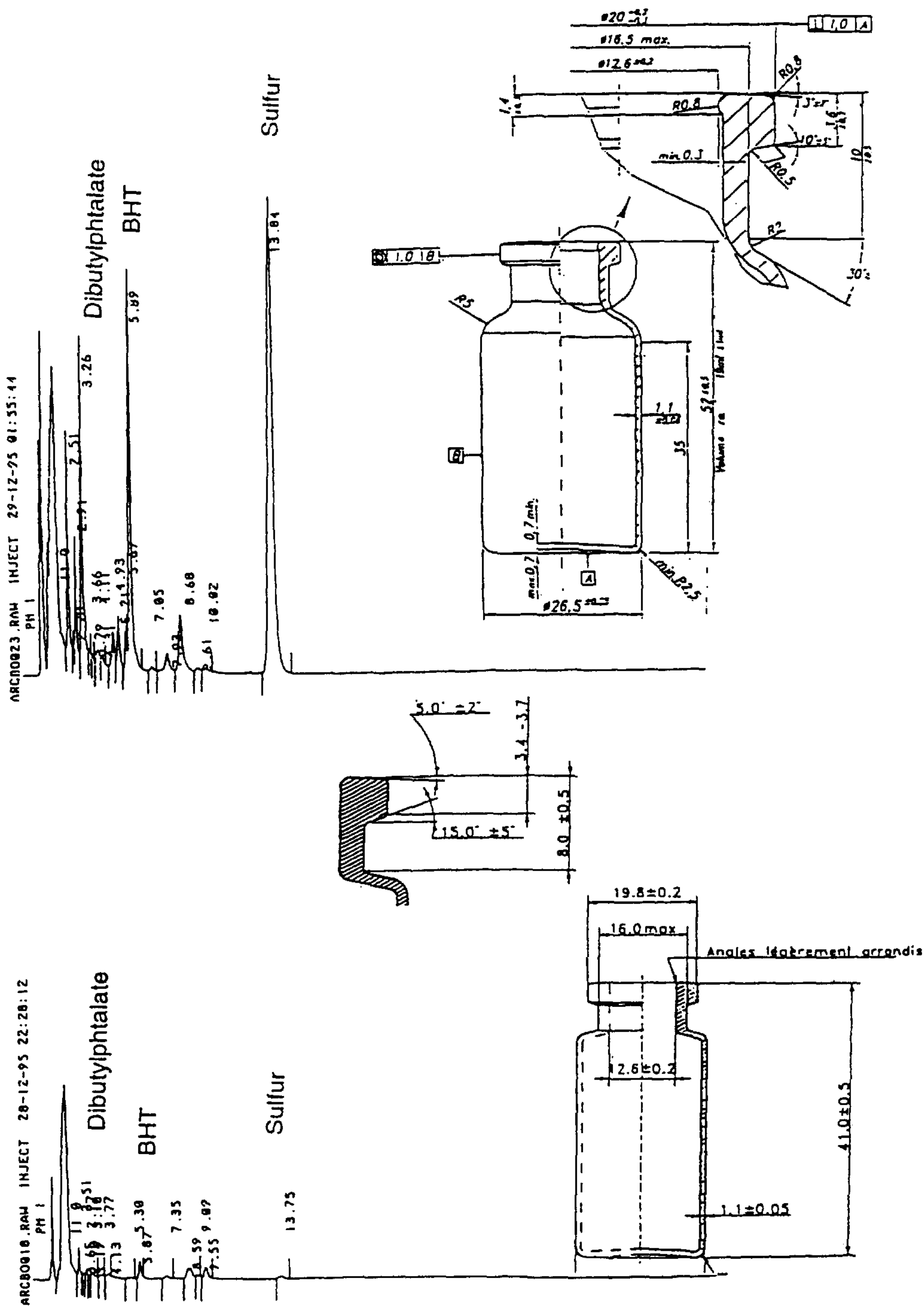

Fig. 10. HPLC analysis of a 1:1 ethanol–water extract (1 month at 65°C) detected by UV absorbance at 230 nm, as a function of the vial design.

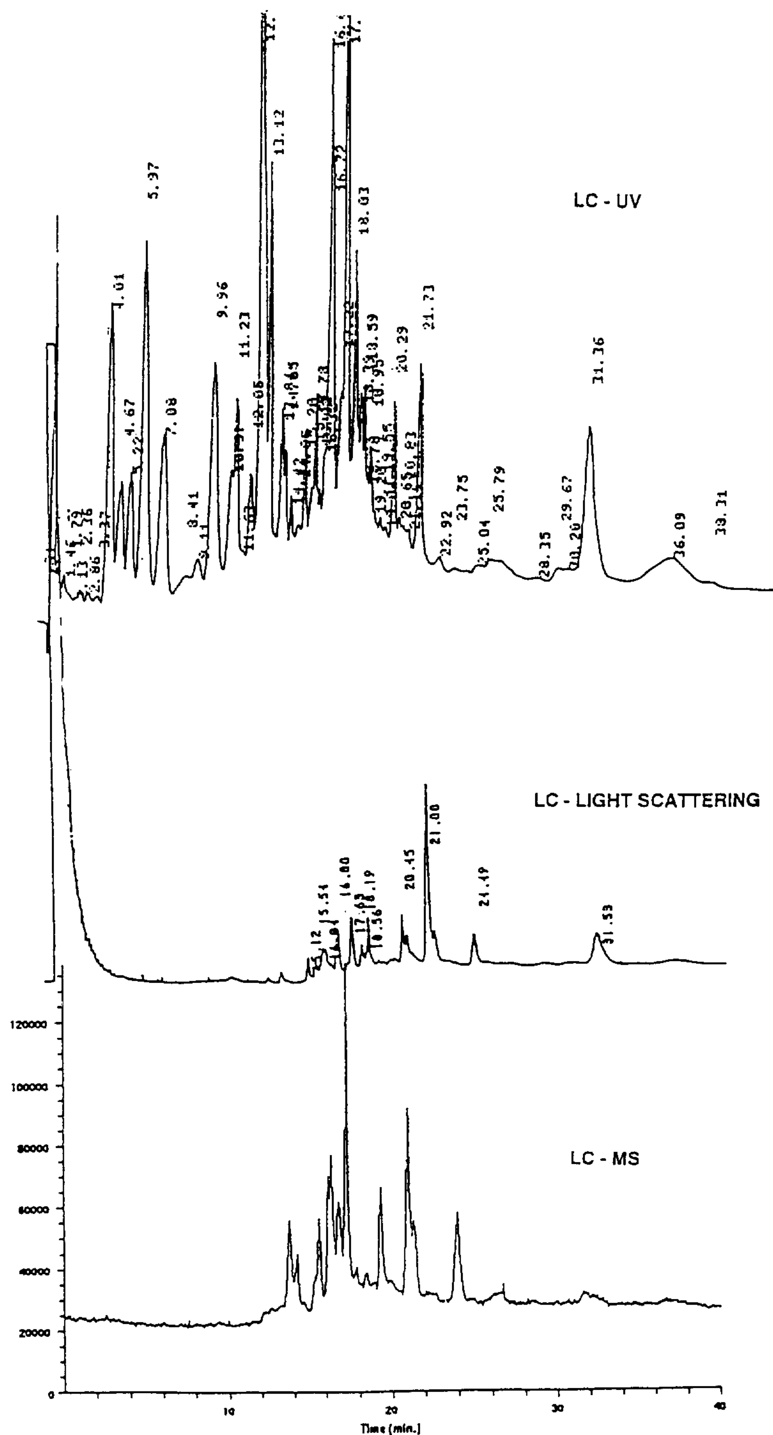

Fig. 11. 1:1 ethanol–water extract of teflonated chlorobutyl rubber stoppers analyzed by HPLC and detected by (top) UV absorbance, (middle) evaporative light scattering detector and (bottom) mass spectrometry with a particle beam interface.

evaluating chemical stability are not more in line with the climatic zones that have been defined in order to facilitate worldwide commercialisation. Further refinements of study conditions will probably be required in the future.

Acknowledgments

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