Drug Purity Profiles

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Abstract □ Physical constants and purity determinations are given for 115 drug substances. Phase solubility analysis, differential scanning calorimetry, and chromatographic data are reported. High-low TLC, which has emerged as a useful, if imprecise, tool for purity approximation, is described. The concept of purity profile is discussed and examples are given; general observations on the utility of methods are made based on cumulative experience.

Keyphrases □ Drug purity profiles—phase solubility analysis, differential scanning calorimetry, and chromatographic data for 115 drugs □ Purity-indicating methods—phase solubility analysis, differential scanning calorimetry, and chromatographic data for 115 drugs □ TLC, high-low—analysis, purity profiles for 115 drugs □ Differential scanning calorimetry—analysis, purity profiles for 115 drugs □ Phase solubility analysis—purity profiles for 115 drugs

As a service to pharmaceutical analysts, useful physical constants and purity profile data are recorded here for samples of 115 drug substances, which were examined as candidates for adoption or continued official recognition as reference standards by either the USP or the NF. General observations on the purity-indicating methods are offered based on extensive experiences with this large number of drug substances. Such broad experience is rare in purity-indicating methodology, and these observations may be of value to analysts in preparing purity profiles.

These are not the only data available to the USP or the NF in connection with evaluation of candidates for recognition as standards. Moreover, these data do not represent the full scope of this laboratory's contribution to the USP and NF programs; notably, no results of monograph tests and assays are reported here. The samples cannot be presumed to have been found acceptable[1] when evaluated here or in the collaborating industrial and governmental laboratories. Only data generated by this laboratory are reported.

METHODS AND MATERIALS

Phase Solubility Analysis (PSA)—Collection and interpretation of PSA data were described fully elsewhere (1). The usual procedure, described in NF XIII (2), consists of charging eight ampuls which are then flame sealed and rotated end-over-end at 25° for 2 weeks. Initial solubility data are obtained occasionally by the method of Reilly and Rae (3), but most often by the approximate method detailed here. Supernates are examined for evidence of decomposition, when deemed necessary, by TLC. Nitrogen-sparged or degassed solvents are used for easily oxidized compounds.

Results from a PSA experiment may be evaluated graphically or by the least-squares best-fit treatment which affords a confidence range (1). Arbitrary confidence classes are assigned (Table I) based partly on that calculation and partly on experienced inspection of the graph. Extrapolated solubility values in pure solvents or in azeotropes are accurate within 0.3 mg./g. for Classes A–C; mixed solvents, however, introduce yet another variable. With respect to the testing strategy, these classes have the following meanings: A or B, almost conclusive or strong evidence of the (im)purity of a sample; C, good evidence, but requires several supporting data; and D, possibly useful as supporting evidence in conjunction with strong alternative evidence. Where an adequate number of tubes[2] is used, a poor confidence rating may reflect either minor solute decomposition in that solvent or inadequate equilibration or drying time. Similarly, solid solutions of the solute with its impurities may be revealed so that an alternative approach is needed. Points near the intersection of the graphical 45° line and the saturation line, *i.e.*, those with sample charges less than 20% above the solubility, occasionally have been found to reflect higher apparent solubility which can lead to purity values in excess of 100%; this can arise from two interrelated phenomena: failure to reach equilibrium and the known higher solubility of fine particles.

Some PSA data listed in Table I were reported earlier (1) from this laboratory but are repeated here to support the evaluation of high-low TLC.

Approximate Solubility—This method is not as rapid as some other approximate methods, but it is much more conservative of the sample, which is often a consideration with high purity drugs. It does not require assay of the solution and is designed specifically to identify solvents for PSA rather than to establish sample solubility in a given solvent. The resultant value is usually ±1 mg./g. of the actual value determined by solubility analysis.

Accurately weigh about 30 mg. of sample into a screw-capped test tube and add three 1.0-ml. increments of the candidate solvent by pipet, mixing on a vortex-causing mixer after each addition. Solubility levels of 30, 15, and 10 mg./ml., respectively, are revealed.

If the sample dissolves immediately in 1 ml. or does not dissolve appreciably in 3 ml., this solvent is ruled out for solubility analysis. If the sample is almost dissolved at any of the three increments, place the stoppered tube in a 25° bath overnight and then observe the extent of solution. At this point, solubility values of about 10, 15, or 30 mg./ml. or values lying between 10 and 15 or between 15 and 30 mg./ml. are identified. Prepare one or two additional tubes, each containing 1 ml. of solvent. The addition of 12.5 mg. of sample will allow definition of solubility in the 10–15-mg./ml. range after standing at 25° overnight; similarly, a 22.5-mg. sample, followed by yet another intermediate sample, allows determination of approximate solubility in the 15–30-mg./ml. range. A total of three tubes is usually sufficient, and analyst experience normally allows even more simplification. Correction for solvent density is not made in selecting sample and solvent weights for charging the PSA tubes.

[1] About 15% of these were not suitable for reference standard usage.

[2] Adequate with respect to the plateau values used in least-squares calculation. The number (n) used for the entries in Table I is coupled with the assigned confidence class. The data are, for practical purposes, univariant and calculated accordingly.

Thermoanalytical Purity[3]—Estimations of purity from melting behavior are made by the modified, integrated Van't Hoff relationship presented by Gray (4) for use with thermograms recorded by differential scanning calorimetry (DSC) instruments. Temperature correction using the slope of the indium endotherm is always applied. Usually the sealed pans offered by the manufacturers are used, but a nitrogen environment is needed for some compounds. Work in this laboratory[4] has revealed no significant or predictable difference in estimates of impurities where thermograms from the two commercial systems were handled in similar fashion. The published baseline corrections are not necessary for these purposes and are not included in the entries in Table I. This same observation was made independently by other workers[5].

In Table I, T_0 is the extrapolated thermodynamic melting point relative to indium. Separate calibration is needed to express these values in terms of the USP–NF melting range because the methods are substantially different. There is no significance to the fact that a given T_0, converted to Celsius, falls within or outside monograph limits.

The ΔH_f values for ultrapure compounds are reproducible (5) with an internal precision of about 2% by quantitative differential thermal analysis (DTA). Few of the compounds here are ultrapure, and it is known that the ΔH_f values obtained depend on the sample purity; therefore, these data are not to be construed as highly accurate thermodynamic constants even though they may be the best values available. For this same reason, values with defined internal precision were not collected. All these values are corrected for premelt and are based on ΔH_f indium = 6.78 cal./g.

Qualitative TLC—Commercial, precoated glass plates or aluminum sheets are used exclusively. Silica gel[6] is the most common adsorbent, but neutral alumina and microcrystalline cellulose (MN-254) are also used. Four systems are developed for each drug substance and, because most are ionizable, the usual practice is to identify both acidic and basic developing solvents. Systems giving R_f 0.3–0.7 for the main spot are preferred. Three visualization techniques are identified. Nonreactive solvents are used where possible[7]. Artifact identification is made by: (*a*) spotting fresh and day-old solutions, and (*b*) two-dimensional TLC, involving wetting of the main spot of the first chromatogram with spotting solvent, drying, turning, and then developing the second chromatogram. Total spots listed in Table I reflect all four or three out of four systems, corrected for artifacts revealed by two-dimensional TLC. This allows reasonable estimation of the number and type of impurities.

General High-Low TLC Procedure—*Sample Solution*—Prepare a sample solution in a suitable solvent at a concentration of about 10 mg./ml. Nonreactive solvents are necessary, preferably degassed or nitrogen sparged. Dilute portions of this solution to concentrations of 1 and 0.5 mg./ml. These directions are general, and occasional solubility problems require their modification. Usually it is possible to apply 250 mcg. or more of drug sample and not exceed 25 μl. sample volume.

Reference Solution—A 10-mg./ml. solution of the specified reference standard in the same solvent is used to compare the identity of subsequent batches of the same drug substance.

Preparation—Divide a suitable TLC plate, 20 cm. square, coated with a 0.25-mm. layer of chromatographic silica gel mixture, into three parts: 2, 2, and 16 cm. in width. Spot 2 μl. of the reference standard solution (if available) in the first division on a line 1.5 cm. from the lower edge of the plate, spot 20 μl. (or largest volume to be spotted) of solvent in the second, and spot volumes equivalent to 250 (200, 100), 10, 5, 3, 2, 1, and 0.5 mcg. of the sample in the last, taking care to obtain comparable starting spots. Allow the plate to develop in a suitable chamber, usually lined with filter paper, which has been allowed to equilibrate at least 1 hr. with the specified developing solvent.

When the solvent reaches a height of 15 cm. from the origin, remove the plate from the chamber and air dry. Locate and compare spots and relative intensities under long- and shortwave UV light. Spray with 0.5% I_2 in chloroform and again rate the relative intensities of spots. Allow the iodine vapors to dissipate. Spray with 40% H_2SO_4 in methanol and view under a UV handlamp. Heat the plate and again view under UV light. For each visualization, estimate the relative abundance of spots as follows: compare each impurity spot in the 250-mcg. sample spotting to the main spots from the 0.5–10-mcg. spottings and assign a value to the relative intensities. For example, 0.4% impurity in the 250-mcg. sample has an intensity equivalent to the 1-mcg. main spot: (1 mcg./250 mcg.) × 100 = 0.4%. A densitometer may be used for these comparisons. Compare the reference standard mobility to that of the 10-mcg. sample spotting as an identity test. In most cases, acid–base indicators, ninhydrin, or Dragendorff sprays are used in this scheme. The 200- and 100-mcg. spottings are useful where there is evidence of overloading at the 250-mcg. level. Smaller spottings often are not visualized by one or more of the methods, so sensitivity estimates must be revised.

High Pressure Liquid Chromatography—An instrument[8] fitted with a gradient elution accessory is used with 1.0-m. × 2.1-mm. stainless steel columns. Commercial, coated packings with chemically bounded phases[9] are used usually with hydroalcoholic eluants in a reversed phase, gradient elution mode. Temperatures between 30 and 50° are employed at pressures up to 80 atm. to obtain flow rates between 0.5 and 1.5 ml./min. Only the low pressure mercury line, 254-nm., detector was used for the drugs reported herein. Drugs were dissolved wherever possible in one of the eluting solvents, 10–30 mg./ml., just prior to injection of 10–100-mcg. samples.

GLC—A temperature-programming instrument[10] is used with flame-ionization detectors. Only the relatively inert methyl and methylphenyl silicones (OV-1, OV-17, and OV-61)[11] on silanized diatomite are used after curing and conditioning, as instructed in the General Tests section of NF XIII (2).

DISCUSSION

The objective of this laboratory's examination of these samples is to allow an independent recommendation to the compendia as to the suitability of a sample for adoption or continued recognition as a standard. To accomplish this goal, it is not deemed necessary to discern precisely and reliably between, say, 0.3 and 0.4% impurity; rather, it is important to recognize samples containing, for example, 1% or greater impurities, to detect an increase in impurities of a few tenths-percent in a sample during storage, or to identify relative differences in impurity contents of subsequent batches of the same drug. Thus, those obvious experimental enlargements leading to more precise values are not pursued once information adequate to the objective is in hand. The data presented here, on the whole, are to be considered as having moderate precision.

Purity estimates should not be based on a single type of data. No method is universally applicable or reliable. Purity values obtained are in different units (mass or mole purity, visualization response, flame-ionization response, 254-nm. absorptivity, *etc.*), and these measurement units can be interconverted only where the identity, properties, and proportions of all impurities are known. Such thorough definition is unusual and seldom valuable and is not the case for the samples listed here.

The individual purity-indicating data converge to establish a profile of purity. It is this profile of purity that is meaningful and that permits decisions about the scope of analytical utility of a given sample.

There is no practical value to reconciling differences between purity-indicating data of moderate precision where both or all methods show the material to be satisfactory or unsatisfactory for the intended uses. Testing strategy and evaluation must begin with a statement of the intended uses of a standard. Different weights are placed on purity profile data according to intended standard usage, *i.e.*, as a chromatographic assay standard as opposed to an IR identity test or a limit test.

Alternative approaches to purity determination are known. Certified contents of the main or minor component by a defined

[3] Two commercial instrumental systems are used in this laboratory, the Perkin-Elmer DSC-1B and the DuPont 990 thermal analysis system.
[4] Drug Standards Laboratory, unpublished comparative study.
[5] D. L. Sondack, T. E. Cole, and J. K. Frishmann, personal communication.
[6] Merck F_{254}.
[7] Acetone reacts with many drugs and is rarely used as a spotting solvent, although it is a common solubility test for identity.
[8] DuPont 820.
[9] Permaphase-Zipax: octadecylsilane (ODS), a saturated hydrocarbon polymer (HCP), and an ethereal polymer (ETH).
[10] Hybrid of H&P 5750B and F&M 810 modules.
[11] OV-61 has since been discarded due to thermal instability.

Table I—Purity Profile Data

Reference Standards USP[a]	Reference Standards NF[a]	Drug Substance[b]	Solubility Analysis: Solvent	Solubility, mg./g.	Impurity, mass %	Confidence Class-n^c	Calorimetry: T_0[d], °K.	ΔH_f[e], cal./mole	Impurities (Premelt), mole %	TLC: Total Spots[f]	TLC: High-Low, Impurity	Impurities Determined by Other Methods[g]
x		Acetanilid	—	—	—	—	385	4900	<0.02	1+ trace	<0.2	—
x		Acetazolamide										
		(*a*)	Acetone	11.1	1.0	B-4	—	—	—	2	0.4	—
		(*b*)	Acetone	10.8	−0.1	D-5	—	—	—	1	—	—
	x	Acetyldigitoxin	Ethanol	15.4	7.65	B-5	—	—	—	3–4	2	Three spots, paper chromatogram; α and β (5%) isomer mixture
	x	Acetyl sulfisoxazole	Chloroform	15.5	0.02	B-6	dec.	—	—	1	—	—
x		Amitriptyline hydrochloride	Acetone	17.9	0.04	C-6	dec.	—	—	1	—	—
x		Amobarbital	—	—	—	—	433	6380	0.08(3.5)	1	—	<0.1%, GLC (C)
	x	Antipyrine	—	—	—	—	382	6570	0.38(9)	1	—	<0.1%, GLC (A)
x		Aspirin	10% ethanol–benzene	14.4	0.18	C-6	—	—	—	1	—	—
	x	Benzocaine	Absolute ethanol	21.8	1.04	C-6	362	5250	0.06(3)	3	0.1–0.2	<0.1%, GLC (A)
	x	Betamethasone										
		(*a*)	Ethanol	18.7	3.7	C-5	dec.	—	—	3	≥2.0	1.4%, HPLC, five peaks (ETH–water, methanol)
		(*b*)	*p*-Dioxane	10.0	3.5	D-5	—	—	—	—	—	—
		(*c*)	Ethanol	19.4	1.3	C-6	—	—	—	3	1.2	0.7%, HPLC, three peaks
	x	Biperiden										
		(*a*)	Ethanol	15.4	0.51	B-8	383	8350	0.07(1)	1	—	Single polymorph
		(*b*)	Ethanol	16.1	6.0	C-5	—	—	>2.5	2	—	Two polymorphs with 2° difference in melting point, DSC (conversion to higher melting with grinding)
	x	Biperiden hydrochloride	Methanol	16.7	1.0	C-5	—	—	—	1	—	—
	x	Bisacodyl	15% chloroform–ethanol	16.3	−0.5	D-6	407	9850	0.15(3)	1	—	—
	x	Brompheniramine maleate										
		(*a*)	Methyl ethyl ketone	28.6	3.0	A-6	406	12,200	0.54(7)	2	3.8	Possible decomposition during DSC
		(*b*)	*n*-Propanol	23.7	3.3	B-5	—	—	—	—	—	—
x	x	Caffeine	—	—	—	—	508	5010	0.05(4)	1	—	No other peaks, GLC (B) and (C)
x		Chloramphenicol	35% ethanol	17.2	0.58	A-6	423	8930	0.19(4)	—	—	—
x		Chlorpheniramine maleate	*n*-Propanol	28.9	0.4	D-5	406	11,400	0.45(5)	2	<1.0	—
x		Chlorpromazine hydrochloride	—	—	—	—	472	6730	0.18(4.5)	4	0.2	—
x		Chlorpropamide	40% chloroform–benzene	9.0	−1.0	C-5	—	—	—	2	0.4	Polymorphism, DSC
x		Cyclizine hydrochloride	Methanol	24.5	0.2	C-5	dec.	—	—	2	0.4	—
	x	Cyproheptadine hydrochloride	40% ethanol	22.4	0.18	A-5	—	—	—	1	—	—
x		Dapsone	10% water–*n*-propanol	22.7	4.28	C-7	451	4710	0.20(4.2)	1	<0.7	—
x		Dexamethasone	Acetone	21.1	0.6	B-6	dec.	—	—	2–3	0.65	—
	x	Dexchlorpheniramine maleate	Ethyl acetate	26.4	1.58	A-15	—	—	—	—	—	14:1 isomer mixture, PSA
	x	Dextromethorphan hydrobromide	Water	20.4	−0.18	A-7	dec.	—	—	1+ trace	—	<0.1%, GLC (B)
	x	Dienestrol	60% ethanol–water	33.6	4.6	C-7	dec.	—	—	5–7	1.4–3.1	—
x		Diethylstilbestrol	—	—	—	—	443	8040	0.36(11)	1+	<0.2	0.1%, GLC (A)
x		Digitoxin										
		(*a*)	Ethanol	14.6	0.46	A-8	—	—	—	2	0.2	—

Table I—(*Continued*)

Reference Standards USP[a]	Reference Standards NF[a]	Drug Substance[b]	Solubility Analysis: Solvent	Solubility, mg./g.	Impurity, mass %	Confidence Class-*n*[c]	Calorimetry: T_0[d], °K.	ΔH_f[e], cal./mole	Impurities (Premelt), mole %	TLC: Total Spots[f]	TLC: High-Low, Impurity	Impurities Determined by Other Methods[g]
		(*b*)	Ethanol	14.6	1.0	C-7	—	—	—	2	<0.3	—
x		Digoxin	95% aqueous methanol	6.13	2.2	A-8	—	—	—	5	2.0	—
	x	Dimethisterone	60% ethanol	11.8	1.11	D-4	—	—	—	2–3	0.4	—
x		Diphenhydramine hydrochloride	—	—	—	—	443	8160	0.42(7)	1	—	0.1%, GLC (B)
x		Diphenylhydantoin	Ethanol	19.4	0.38	D-6	—	—	—	1	—	0.6%, GLC (A)
	x	Dromostanolone propionate	5% water–methanol	27.8	1.2	B-4	403 dec.	5040	0.56(10)	2–3	1.1	—
	x	Dydrogesterone	Isopropanol	19.5	0.01	A-6	445	6800	0.14(4)	1+	—	—
	x	Ephedrine sulfate	Ethanol	15.0	1.34	A-6	—	—	—	1	—	—
	x	Estradiol	—	—	—	—	453	6880	0.14(3)	2	0.1–0.2	Polymorphism, DSC; <0.1%, HPLC (ODS/water–isopropanol)
	x	Estradiol benzoate (*a*)	—	—	—	—	471	8780	0.26(5)	3	0.7/0.6 +0.1	0.12% (estradiol), GLC (B); 0.5%, HPLC, (ODPN 5% tetrahydrofuran in isooctane)
		(*b*)	—	—	—	—	470	8570	0.71(7.5)	—	—	—
	x	Estradiol cypionate	—	—	—	—	427	7030	0.10(4)	3	0.8	≤0.9% (estradiol), HPLC [ODS/methanol–water (2:1)]
	x	Estradiol dipropionate (*a*)	Ethanol	18.2	0.83	A-6	375	7170	0.55(10)	3	1.0	Four species, one impurity, 15% a/a, HPLC, (HCP, 55% methanol); 2.1%, GLC (B)
		(*b*)	—	—	—	—	376	6150	0.17(3.5)	3	2.3	91.5% drug, 7% main impurity, HPLC, (HCP, 55% methanol); 2.8%, GLC (B)
x		Estradiol valerate	80% ethanol–water	16.8	1.00	A-6	422	7010	0.27(6.5)	2	0.5	—
x	x	Estrone	Acetone	14.6	0.8	C-6	—	—	—	2–3	—	No other peaks, GLC (B)
x		Ethacrynic acid	—	—	—	—	396 dec.	(7860) (7130)	0.17(5) 0.38(7)	2	0.5	—
x		Ethinyl estradiol (*a*)	—	—	—	—	459	6610	0.20(7)	2	0.4	No other UV 254 peaks (ETH-Zipax, methanol, pH 4.7 buffer); 1.0%, GLC (B)
		(*b*)	—	—	—	—	454	5350	1.50(13.5)	5	—	10 UV 254 peaks
x		Ethionamide	Acetone	23.7	−0.14	C-5	—	—	—	1+ trace	—	—
	x	Ethisterone	Chloroform	6.4	−0.3	B-6	dec.	—	—	1	—	One peak, HPLC (ETH/water, methanol)
x		Ethoxzolamide	—	—	—	—	464	9370	0.30(10)	1	—	—
	x	Fluocinolone acetonide	60% ethanol–water	10.9	0.76	B-6	—	—	—	1	—	—
x		Fluoxymesterone	Ethanol	14.3	0.3	B-5	dec.	—	—	1	—	—
	x	Fluphenazine hydrochloride	Ethanol	29.3	0.59	D-5	—	—	—	2–3 trace	0.6	—
	x	Flurandrenolide	60% ethanol–water	21.9	−0.3	D-6	—	—	—	1+ trace	—	—
x		Furosemide	Ethanol	17.1	−0.3	D-6	—	—	—	2–3	<0.2	—

(*continued*)

Table I—(*Continued*)

Reference Standards USP[a]	Reference Standards NF[a]	Drug Substance[b]	Solubility Analysis: Solvent	Solubility, mg./g.	Impurity, mass %	Confidence Class-n^c	Calorimetry: T_0^d, °K.	ΔH_f^e, cal./mole	Impurities (Premelt), mole %	TLC: Total Spots[f]	TLC: High-Low, Impurity	Impurities Determined by Other Methods[g]
	x	Glutethimide	40% ethanol	13.3	0.8	B-5	358	4760	0.25(7)	1+ trace	—	—
x		Hexachlorophene	—	—	—	—	437	8040	0.08(4)	1	—	No other peaks, GLC of trimethylsilyl derivative (B)
x		Histamine dihydrochloride	4% water in *n*-propanol	15.5	−0.5	C-6	dec.	—	—	1	—	—
x		Hydrocortisone	Ethanol	27.8	1.4	D-5	—	—	—	1	—	None, quantitative TLC; 32% polymorph, PSA
x		Hydrocortisone acetate	*p*-Dioxane	9.7	0.6	C-6	dec.	—	—	1	—	0.1%, quantitative TLC
	x	Indomethacin	Ethanol	24.8	0.64	C-5	433	9000	0.12(4)	1+ trace	<0.2	—
	x	Isopropamide iodide	Water	21.6	1.28	C-6	dec.	—	—	3	1.0–1.5	—
	x	Lanatoside C	Absolute ethanol	19.8	23.0	C-6	dec.	—	—	2–3+	—	Glycoside mixture
	x	Menadione	—	—	—	—	376	5130	0.15(5)	—	—	—
x		Meprobamate	—	—	—	—	376	8950	0.40(8)	1	—	1 peak, GLC (A)
x		Mestranol	Ethanol	21.1	0.40	B-6	427	8450	0.09(1.5)	—	—	<0.2%, GLC (B)
	x	Metabutethamine hydrochloride	Isopropanol	26.4	−0.56	A-5	dec.	—	—	1+ trace	—	0.1%, GLC (A, B)
x		Methamphetamine hydrochloride	Isopropanol	32.1	1.11	B-5	447	6370	0.45(12)	1+ trace	—	0.4%, GLC (A)
	x	Methantheline bromide	*n*-Propanol	28.8	1.25	C-6	dec.	—	—	—	—	—
	x	Methdilazine hydrochloride	Acetone	17.6	1.16	D-6	dec.	—	—	3–4	<0.6	—
x		Methimazole	Ethyl acetate	28.6	−0.5	A-6	—	—	—	1	—	—
	x	Methohexital	Ethanol–water (1:1)	13.3	0.39	B-5	365	6740	0.15(4)	1	—	—
	x	Methyclothiazide	Methanol	19.6	−0.05	A-6	—	—	1	1	—	—
	x	Nandrolone phenpropionate	—	—	—	—	367	5700	0:63(7)	1	—	0.9%, quantitative TLC
x		Neostigmine bromide	*n*-Propanol	24.8	−0.04	B-5	dec.	—	—	1	—	—
x		Neostigmine methylsulfate	*n*-Propanol	30.0	0.00	D-4	423	7140	0.04(2)	1	—	—
x		Niacinamide	Ethyl acetate	10.9	−0.9	C-7	402	5460	0.14(4)	1	—	—
	x	Nialamide	40% ethanol	24.9	−0.2	C-5	427	10,920	0.15(1.5)	1	—	—
x		Nitrofurantoin	20% dimethylformamide–acetone	21.6	−0.36	B-6	—	—	—	1	—	One peak, HPLC (SAX/0.1 *N*, pH 9.1 borate buffer)
	x	Norethandrolone	50% ethanol	13.3	4.77	B-6	412	5100	0.13(3)	up to 6	≤3.0	0.2%, GLC (B)
x		Norethindrone	Acetone	19.5	1.38	A-7	481	8740	0.20(5)	1	—	—
	x	Norethindrone acetate	60% ethanol	13.8	1.33	C-5	438	6500	0.49(14.7)	3–5	<1.0	About 9% polymorph, PSA
x		Norethynodrel										
		(*a*)	30% acetone–hexane	21.2	1.68	C-6	459 dec.	6550	1.57(0)	5	<1.5	Up to 3.6% added norethindrone not revealed by DSC; mestranol revealed by DSC
		(*b*)	30% acetone–hexane	20.8	2.95	D-5	455 dec.	7020	1.80(10.5)	3–4	—	—
		(*b*)	Isopropanol	24.6	2.62	D-5	—	—	—	3–4	—	—
		(*c*)	30% acetone–hexane	20.9	−0.01	B-11	—	—	—	1	—	—
	x	Oxandrolone	Acetone	17.3	0.66	A-6	dec.	—	—	2	<1.0	—
x		Phenacetin	Ethyl acetate	23.1	0.40	C-4	410	7480	0.08(8)	2	<0.2	—
	x	Phenazopyridine hydrochloride	Pyridine	10.4	−0.10	B-5	—	—	—	3	0.6	—

Table I—(*Continued*)

Reference Standards USP[a]	Reference Standards NF[a]	Drug Substance[b]	Solubility Analysis: Solvent	Solubility, mg./g.	Impurity, mass %	Confidence Class-n^c	Calorimetry: T_0[d], °K.	ΔH_f[e], cal./mole	Impurities (Premelt), mole %	TLC: Total Spots[f]	TLC: High-Low, Impurity	Impurities Determined by Other Methods[g]
x		Phenformin hydrochloride	—	—	—	—	451	7902	0.06(2)	1	—	—
	x	Phenprocoumon	90% ethanol	31.8	0.59	A-6	456	8530	0.37(7)	1	—	—
x		Phenylbutazone	—	—	—	—	377	7090	0.25(8)	2	<0.2	—
x		Pralidoxime hydrochloride	Ethanol	33.6	0.46	D-5	—	—	—	2	0.1	—
x		Prednisolone acetate										
		(*a*)	*p*-Dioxane	13.5	1.40	D-5	—	—	—	1	—	0.3%, quantitative TLC
		(*b*)	*p*-Dioxane	13.5	4.94	B-5	dec.	—	—	2	1.7	0.5–1.5%, quantitative TLC
x		Prednisone	Ethanol	8.5	0.10	B-5	dec.	—	—	1	—	—
x		Procaine hydrochloride	—	—	—	—	429	8610	0.04(1.5)	1	—	—
	x	Progesterone	—	—	—	—	403	6140	0.06(1)	1–2	<0.1	0.6%, HPLC (ODS/10–40% isopropanol in water)
	x	Promazine hydrochloride	Isopropanol	20.6	0.13	C-12	455	7710	0.20(3)	2	0.2	—
x		Propoxyphene hydrochloride	*p*-Dioxane	25.6	1.52	B-7	—	—	—	1	—	—
x		Pyrazinamide	—	—	—	—	463	6790	0.10(3.2)	1	—	—
x		Pyridoxine hydrochloride										
		(*a*)	Ethanol	13.2	0.80	A-6	dec.	—	—	1	—	—
		(*b*)	Ethanol	13.4	1.6	C-7	—	—	—	3	<0.2	—
	x	Pyrilamine maleate	Ethyl acetate	11.2	−0.03	B-10	—	—	—	2	≤0.6	—
x		Resorcinol	1% ethanol–chloroform	8.2	0.60	C-6	382	4750	0.04(3)	1–2	—	—
	x	Saccharin	Isopropanol	21.4	−0.37	C-4	499	6550	0.32(5)	1+ trace	—	—
	x	Salicylamide	40% ethanol	25.5	−0.07	C-5	414	6390	0.02(1)	1+ trace	—	None, GLC (B) 0.5%, GLC (A)
x		Scopolamine hydrobromide	Absolute ethanol	20.0	−0.3	C-6	dec.	—	—	1	—	0.4%, GLC (A)
x		Spironolactone	60% ethanol–water	16.9	0.4	A-8	dec.	—	—	2	0.1	None, HPLC (ODS, 55°, 10% *n*-propanol–water)
x		Sulfapyridine	—	—	—	—	466	8730	0.23(4)	2	0.2	—
x		Sulfisoxazole	Ethyl acetate	15.4	−0.14	C-5	dec.	—	—	2	<0.4	—
	x	Testosterone	—	—	—	—	429	6250	0.33(1.5)	3–4	<0.3	None, HPLC (ETH/water, isopropanol); 1%, HPLC (ODS/10% *n*-propanol in water)
x		Testosterone cypionate	70% methanol	1.80	0.10	A-7	373	5640	0.13(5)	1	—	—
x		Testosterone enanthate										
		(*a*)	—	—	—	—	312	4960	1.47(15)	6	1.5	1.7%, HPLC, six peaks, (ODS/30% isopropanol in water)
		(*b*)	—	—	—	—	312	5920	1.08(8.5)	6	0.7	—
x		Tetracaine hydrochloride	40% absolute ethanol–isopropanol	11.6	−0.3	B-6	dec.	—	—	2	0.2	—
x		Thiabendazole	Methanol	11.3	0.82	B-6	dec.	—	—	1	—	—
x		Thiopental	10% ethyl acetate	20.7	0.50	D-6	431	7010	0.17(5)	1	—	—
x		Tolbutamide	Benzene	15.6	0.50	A-5	400	6300	0.20(5)	1+ trace	—	—
x		Tolnaftate	—	—	—	—	384	7350	0.72(11)	2+ trace	<1.0	—
	x	Tranylcypromine sulfate	70% ethanol	25.6	1.0	C-5	—	—	—	1	—	—

(*continued*)

Table I—(*Continued*)

Reference Standards			Solubility Analysis			Calorimetry			Impurities (Premelt), mole %	TLC		Impurities Determined by Other Methods[g]
USP[a]	NF[a]	Drug Substance[b]	Solvent	Solubility, mg./g.	Impurity, mass %	Confidence Class-n[c]	T_0[d], °K.	ΔH_f, cal./mole		Total Spots/ Impurity	High-Low, Impurity	
x		Triamcinolone acetonide	Acetone	20.0	0.29	B-7	—	—	—	23	1.7	Triamcinolone not revealed by PSA
	x	Triamcinolone diacetate	70% ethanol	13.2	−0.78	B-5	—	—	—	1+ trace	—	—
x		Trihexyphenidyl hydrochloride	20% ethanol	14.6	0.62	C-5	dec.	—	—	2	<0.4	—
x		Trimethaphan camsylate	Isopropanol	24.5	0.1	C-6	dec.	—	—	1+ trace	—	—
	x	Trioxsalen	Chloroform–methanol (2:1)	25.6	0.8	D-4	508	6790	0.51(8)	3–4	<0.6	—
x		Tripelennamine hydrochloride	—	—	—	—	459	8490	0.37(7)	2	<0.5	—
x		Tropicamide	20% ethanol	23.4	0.8	D-6	368	8990	0.10(2.5)	1	—	No other peaks, GLC (B) and (C)
x		Tubocurarine chloride	Ethanol	30.0	0.29	B-6	dec.	—	—	3	~0.5	*d*-Isomer
x		Vanillin	—	—	—	—	353	(5100)	0.06(2)	1	—	Sublimes, ΔH varies
	x	Vinbarbital	—	—	—	—	439	7270	1.52(12.5)	1	—	0.9%, GLC (A)

[a] Samples cannot be presumed to have been found acceptable by the responsible compendial body. [b] *a*, *b*, and *c* represent different batches. [c] Confidence classes are defined as: A or B, almost conclusive or strong evidence of the (im)purity of a sample; C, good evidence but requires several supporting data; and D, possibly useful as supporting evidence in conjunction with strong alternative evidence. n = number of tubes used. [d] T_0 = extrapolated thermodynamic melting point related to indium. [e] Values are based on H_f indium = 6.78 cal./g. [f] Total number of impurities as determined by qualitative TLC. [g] For GLC systems: A, 3% OV-17 on 100–120-mesh Gas Chrom Q; B, 3% OV-1 on 100–120-mesh Gas Chrom Q; and C, 3% OV-61 on 80–100-mesh Gas Chrom Q. HPLC = high pressure liquid chromatography. ETH = an ethereal polymer; DSC = differential scanning calorimetry. PSA = phase solubility analysis. ODS = octadecylsilane. OPDN = β,β'-oxydipropionitrile. HCP = a hydrocarbon polymer.

method are necessary for such applications as primary titrimetric, calorimetric, or trace standards[12]. Exhaustive definition of the identity and quantity of every impurity in a given lot of drug standard has been achieved elsewhere for a few compounds, but this approach has been judged unsatisfactory and uneconomical[12]. Practical evaluations of a few ultrapure reagent samples[14] and the strategies involved were discussed recently (6).

Profile Interpretations[15].—Estradiol dipropionate was evaluated by PSA, DSC, TLC, and high pressure liquid chromatography. The material was proposed for use partly as a reference standard in a UV assay. Solubility analysis showed 0.8% mass impurity, and scanning calorimetry revealed 0.5 mole % eutectic impurity. High-low TLC and GLC gave a 1.0–1.2% impurity level. At this point, the material could be used as a standard for IR or thin-layer identification purposes. However, liquid partition chromatography revealed six components and that one of the impurities was intensely UV absorbing, about 15% of the area under the curve at 254 nm. Another sample showed 7% in the 254-nm. data but more mass impurities. Collection of the main impurity showed that it interfered about 6 and 3%, respectively, at the monograph wavelength of 268 nm. Therefore, the material was unacceptable as a UV assay standard. *This is a striking illustration of the necessity of interpreting purity profile data in light of the intended uses of a standard.*

Betamethasone was evaluated by liquid partition chromatography; about 1.4% UV absorbing impurity was evident. Quantitative TLC showed only 0.5% and high-low TLC indicated 1.0% impurity. Thermal decomposition precluded an estimate of eutectic impurity. Solubility analysis in a polar alcoholic solvent revealed that the main component was only 96.3% pure. Because polymorphism could account for a discordant solubility analysis, especially for a steroid, a second solubility analysis, this time in an ethereal solvent, was performed. The result, 96.5%, was confirmative, excluding polymorphism as an explanation for the data. The presence of process-related, saturated steroids was suspected, because these would not absorb UV light and, therefore, would not be revealed by either quantitative TLC (recovered *versus* original) or the 254-nm. detector; less detectability is anticipated with most visualization reagents so that the high-low value would register less than the mass present. A subsequent batch assayed 98.7% by solubility analysis, high-low TLC revealed 1.2% impurity, and liquid chromatography showed only 0.7% impurity. The UV absorbance at the monograph wavelength was about 1% greater than that of the former batch, which partially confirmed the interpretation.

Acetanilid was marked for use as a melting-point standard. The material was chromatographically homogeneous. Calorimetric purity analysis indicated that less than 0.02% eutectic impurities could be present, the most desirable feature for a melting-point standard. On the other hand, estradiol cypionate was shown to contain about 0.8% impurity, mostly estradiol, by chromatography, whereas eutectic impurities registered only 0.1%. Such discordance of calorimetric purity with other methods is particularly severe in steroids where solid solutions are commonplace.

Norethynodrel was evaluated by solubility analysis, calorimetry, and TLC. A three- or four-component mixture was indicated by TLC, and a 1.8% eutectic impurity was in evidence. Solubility analyses in two solvent types both demonstrated a high level of impurity, about 2.7%. A subsequent batch showed unusual correlation of PSA, DSC, and high-low TLC values at the 1.6% level which, for a compound of this type, may be currently the best achievable purity.

Dexchlorpheniramine maleate was found by solubility analysis to contain a less soluble component (13.8%) and soluble impurities (1.6%). The 13.8% component was interpreted as the racemic compound due to the presence of a 6.9% *l*-isomer. The solubility of the racemic compound (4.5 mg./g.), allowing for mass action due to maleate, was consistent with the value predicted from the phase diagram, and chromatographic data also were consistent with this interpretation.

[12] The National Bureau of Standards is the major source of such standards.

[13] C. A. Johnson, British Pharmacopoeia, personal communication.

[14] Ultrapure reagents are needed mostly in trace and inorganic analyses.

[15] Only the specialized purity-indicating data are listed here; monograph tests were performed on each, such as moisture content, spectral characteristics, and optical rotation.

Most of the profiles in Table I are straightforward, and the above examples were chosen to illustrate either the interpretations of discordant data or the variable weight assigned to data with respect to the intended uses of a standard.

COMMENTS ON METHODS

PSA—Procedures and interpretation of results were discussed in detail elsewhere (1, 2, 7, 8). This laboratory has made available systems for 120 drugs [including those of an earlier summary (1)]. Approximate solubilities may not be close enough to the PSA-determined value, particularly where substantial impurities exist, so that additional tubes may be required at a later time to fill in the phase diagram. This open-ended feature of PSA is valuable. Where greater precision or definition is required, more tubes may be added. More importantly, additional solvents or temperatures may be chosen. Indeed, identical results of solubility analyses in two chemically different solvents may be taken as conclusive purity evidence.

PSA is the most generally applicable absolute purity determination. Scrupulous attention must be given to technique and choice of systems. A disadvantage of the method is the lengthy elapsed time[16] before interpretation is possible. Solid solutions (9) and disproportionation of salts (10) are encountered occasionally, but recent modifications show promise of extending the scope of solubility analysis to these difficult situations, Successful analysis (10) of atropine sulfate has been reported using a picric acid system, whereas probable disproportionation has prevented analysis in this laboratory by the usual procedure. Apparent polymorphism in the solvent is a more common problem, particularly with steroids, and this may explain the data for hydrocortisone, norethindrone acetate, and prednisolone acetate. A change of systems would be indicated where other purity profile information does not allow interpretation.

High-Low TLC—The need for a semiquantitative TLC test, one that could be used to validate precise but unselective assays such as titrations, has existed for some time. Obvious theoretical arguments against the accuracy of such a test can be overwhelming; nevertheless, it is one purpose of this report to prove the practical value of one such procedure by comparison of the results of (im)-purity estimates with values obtained by more definitive methods. High-low TLC has been instituted in this laboratory out of a suggestion from Johnson[13]. The need for such a test arose from the reference standard evaluation program, in which samples containing minor impurities must be evaluated without benefit of comparison to other standards. This situation is critical in instances where neither solubility nor calorimetric analysis has yielded interpretable purity data.

Typically, varying sample sizes are spotted on a single plate, the chromatogram is developed, and unselective visualization methods are used. Impurity spots in the heaviest spotting are compared in intensity and size to the main spot in each of the smaller sample applications, and the estimated impurities are summed for an overall estimate. The limiting assumption here is that the drug samples are contaminated with chemically related materials. Even with related materials, responses vary so that the usual practice is to use three unselective visualization methods. This method is a realistic approach only where a few percent of impurities are anticipated.

High-low TLC is most useful exactly where quantitative (by extraction) TLC is not reliable, such as distinguishing between 0.5 and 1.0% impurity and ranking subsequent batches of the same drug. TLC data, in general, have not been useful for materials having less than 0.2% impurities.

Correlation of high-low results in Table I with PSA and DSC data shows that this method is reasonably reliable, usually within 0.5% of the total impurity, with PSA–TLC being better correlated than DSC–TLC. High-low TLC and qualitative TLC are valuable tools in constructing purity profiles, and the use of these procedures in conjunction with DSC is recommended strongly for validation.

Other Chromatography—Qualitative and quantitative TLC evaluation of drug purity and stability is commonplace and requires no discussion. It is important to note that recovery and precision considerations easily obscure purity information, particularly where an established standard is not available for direct comparison. In quantitative thin-layer work, the bias is usually in the direction of underestimating the recovered species, and assays are of little value when a standard of known purity is not available.

GLC purity estimates play only a minor role in initial establishment of, as opposed to comparative assay with, a reference standard. GLC purity alone is valid only for distillates, because only the volatile portion of the sample is eluted for measurement. Similarly, only the least polar phases are of value in general purity work. Multiple products are not unusual with derivatization, and any reaction or extraction can constitute a purification step. Thermal-injection artifacts are all too common but are easily distinguished from impurities by comparison of chromatograms resulting from stepwise variation of injection port temperature from that of the column to 150° hotter ("hot-port" experiment).

High-pressure liquid chromatography has substantial potential for purity-indicating data, but applications of this technique are too recent to allow many generalities. At present, it appears that this recent instrumental development, along with excellent new packing materials, has made available a major purity test. Separate evaluation of this tool is planned.

DSC—As a first proposition, this method cannot be regarded as ever yielding absolute estimates of (im)purity. However, where prudently applied, this method can be of great value in drug purity evaluation. Combined with strong supporting evidence such as TLC, the method is well suited to purity and stability evaluations. The method is capable of considerable precision and reproducibility. Discussions of the experimental details and scope are available (5, 11–14). Reliance on this method requires at least the exact knowledge of the history of a sample and the types of likely impurities. Precalibration against other purity-indicating information and use of defined standards are preferred. Most drug substances are not amenable to calorimetry because of thermal decomposition during melting or, in certain situations, significant vapor pressure. Experience with more than 150 compounds, randomly received, indicates that about 30% of official drug substances yield calculable thermograms. The problem of low ΔH_f compounds was recognized earlier (13). In these cases, and for solid solutions as well, melting-point phase diagrams, as discussed by Marti *et al.* (14), may be of value, but samples with proven purity are needed to construct the diagrams. All these remarks apply only to quantitative DSC and not to the major value of thermal analysis for such phenomena as polymorphism.

Several distinctive problems should be discussed. Only eutectic impurities are revealed; solid solutions lead to impurity estimates that are too low. The thermogram does not reveal solid solutions (12). For example, the presence of 3.6% added norethindrone in norethynodrel was not measured by DSC, although this is measurable by UV spectrophotometry; in contrast, the presence of added mestranol was revealed by DSC.

Correction for the fraction of sample melted prior to instrumental response is a feature of the usual DSC method (11–13). This empirical correction (and resulting slope and impurity value) depends on an arbitrary choice of those areas to be used in the temperature *versus* reciprocal fraction-melted plot. For example, varying the choice of areas in the phenacetin analysis resulted in premelt factors ranging from 1 to 15% and in impurity values ranging from 0.02 to 0.40 mole %.

The T_0 and ΔH_f values are affected somewhat by the random selection of areas, but minor variation in these values has negligible effect on the calculated impurity. The premelt correction is varied until the theoretical linear relationship is visually obtained in the plot. However, many drugs yield S curves in the plot, which cannot be even approximately linearized. Premelt correction then is entirely arbitrary, allowing for great variability in the final impurity calculation. Such was the case with diphenhydramine hydrochloride, ethacrynic acid, hexachlorophene, menadione, methamphetamine hydrochloride, norethindrone acetate, norethynodrel, phenacetin, and phenprocoumon. For example, impurity values between 0.2 and 0.4 mole % could have been reported for phenprocoumon because of visual uncertainty in determining the best line for the data. The values reported[17] in the table for these drugs represent results obtained using the premelt correction yielding the least-squares best fit with the lowest standard error. A three-point estimate is

[16] A vibrational method is used elsewhere but has not been found to be reliable here except in instances of large sample charges.

[17] Calculated by means of a programmable desktop calculator.

also used (15). In cases where S character is strong, we recommend that impurity values be calculated using several premelt correction values as an indication of the confidence. Computer programs that calculate a single value for impurity based on a single best fit should be used with caution, as the degree of S character is not specifically determined even though the premelt correction is well controlled. Studies on the impact of this problem are in progress. The magnitude of the premelt correction in Table I correlates roughly with the level of impurity, as should be expected: 0.0–0.2 mole % impurities averaged 3.3% premelt correction, 0.2–0.5 mole % averaged 6.8% premelt correction, and >0.75 mole % averaged 11% premelt correction. A sample that generates a premelt correction not consistent with this trend should be examined more carefully by other methods before such purity data are interpreted.

SUMMARY

Purity constants and analytical systems are reported for samples of 115 official drug substances analyzed by various combinations of PSA, DSC, high-low TLC, GLC, and high pressure liquid chromatography. General experiences are discussed for these purity-indicating techniques. No one method is universally reliable or applicable, and purity decisions should be based on the largest variety of data available.

The individual purity-indicating data on a given sample converge to establish a profile of purity. It is this purity profile that is meaningful and that allows decisions about the scope of usefulness of the sample as a reference standard. Examples of interpretations are given for a few of the purity profiles.

PSA continues to be the most valuable and generally applicable purity technique. DSC has value in the construction of purity profiles, but it can never stand alone and is applicable to only a minority of drugs. GLC is of minor value in establishing purity profiles. On the other hand, high pressure liquid chromatography has demonstrated a potential for major value in purity work.

Despite the inherent inaccuracies in high-low TLC, a direct correlation exists in the majority of cases between the values obtained by PSA and this method. It is possible, where a rapid method is needed, to determine approximate purity within a day using high-low TLC, especially where a marked correlation exists between purity estimates determined by the methods discussed here.

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