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Assay and purity evaluation of sunepitron hydrochloride by reversed-phase liquid chromatography using a reference standard composite[1]

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Abstract

A reference standard composite was prepared that contained the active pharmaceutical ingredient sunepitron and three potential impurities. This standard was characterized and used for concomitant quantitation of sunepitron and its potential impurities in samples of drug substance and drug product. This approach minimizes the number and quantity of reference standards which often are expensive to synthesize, characterize, and maintain. In addition, running assays becomes simpler because the number of reference standard solutions required for each assay is reduced. Reference standard composites can also be used for qualitative applications such as demonstrating system suitability or for retention times markers for process related impurities or degradants. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Sunepitron; Reference standard composite; Reversed-phase liquid chromatography; Pharmaceutical assay

1. Introduction

Sunepitron hydrochloride is a drug candidate under development for the treatment of anxiety and depression. The chromatographic methodology developed to determine the potency and purity of sunepitron hydrochloride drug substance and tablets has been reported [1,2]. This methodology allows concomitant quantitation of the main component (sunepitron) and potential impurities from the same sample solution. This approach of combining purity evaluation and assay of the main component saves the time and resources of analytical laboratories. To further improve efficiency, a reference standard composite containing sunepitron and three potential impurities was prepared. This paper describes the preparation, characterization and application of a reference standard composite for the assay and purity evaluation of sunepitron hydrochloride. Although there have been several recent reports

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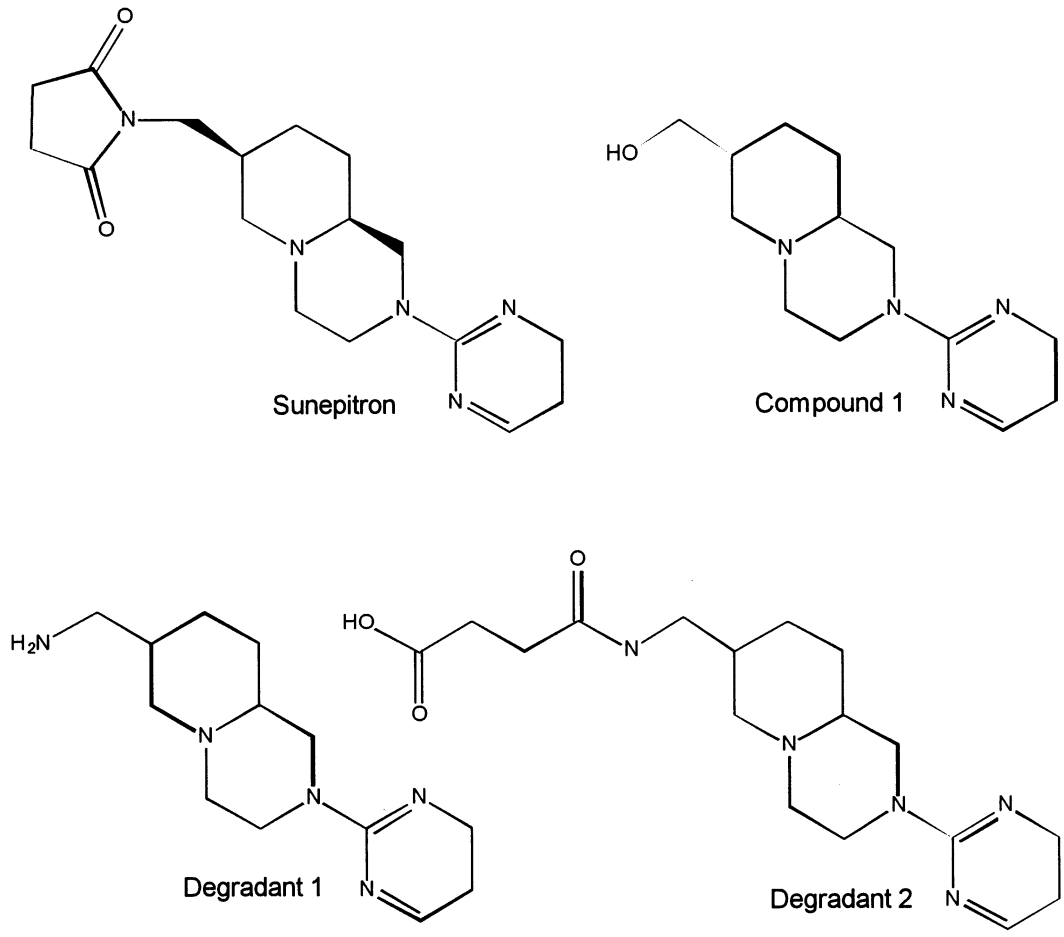

Fig. 1. Chemical structures of sunepitron, compound 1, and degradants 1 and 2.

describing purity evaluations of pharmaceuticals, none of these employ reference standard composites [3–6].

By combining several compounds into one reference standard, several advantages are obtained. Since only small quantities of the spiked impurities are required to prepare the final reference standard composite, the resource and financial commitments of the synthesis laboratories are minimized. Reference standards of impurities may be much more difficult to supply in large quantities (ca. 100 g) compared to a reference standard of the active drug substance whose synthesis has been thoroughly studied, developed, and optimized. It is possible that resources may be further saved by selecting a batch of drug substance that already contains some of the impurities of interest and then adding the remainder.

The resource commitment for characterization of reference standards also can be reduced because fewer standards are required that need extensive characterization. Initial characterization of a reference standard composite, however, is more involved than for single compound standards, because of potential problems with stability and content uniformity.

By combining standards, the handling properties (e.g. hygroscopicity) of individual standards can improve. The greatest benefit of combining

Table 1
Content uniformity data for the reference standard composite

Sample	Sunepitron (%)	Degradant 1 (%)	Compound 1 (%)	Degradant 2 (%)
1	95.8	0.23	0.21	0.77
2	97.4	0.21	0.18	0.68
3	96.0	0.23	0.22	0.79
4	96.0	0.23	0.21	0.78
5	96.2	0.23	0.23	0.80
6	95.4	0.23	0.23	0.80
7	96.7	0.23	0.22	0.79
8	95.9	0.23	0.20	0.78
9	95.8	0.23	0.20	0.77
10	95.8	0.24	0.26	0.83
Average	96.1	0.23	0.22	0.78
R.S.D. (%)	0.6	3.2	10	5.0

Date of analysis 3/1/96.

standards is the improvement in long-term efficiency by the analytical laboratories that run the methods. Since each solution of the reference standard composite contains several components in a fixed ratio, the time and potential errors associated with sample preparation are minimized. In addition, chromatographic analysis time also decreases because all standards (impurities and parent) are injected simultaneously. The need to prepare and inject additional standards to demonstrate system suitability or identify impurity peaks is also eliminated. Finally, fewer standard solutions and shorter chromatographic runs result in a reduction in the quantity of solvents consumed.

2. Experimental

2.1. Materials

Individual standards of sunepitron hydrochloride and its potential impurities (Fig. 1) were prepared and characterized at Pfizer (Groton, CT). Sunepitron is the free base form of the compound; sunepitron hydrochloride is the hydrochloride salt of sunepitron.

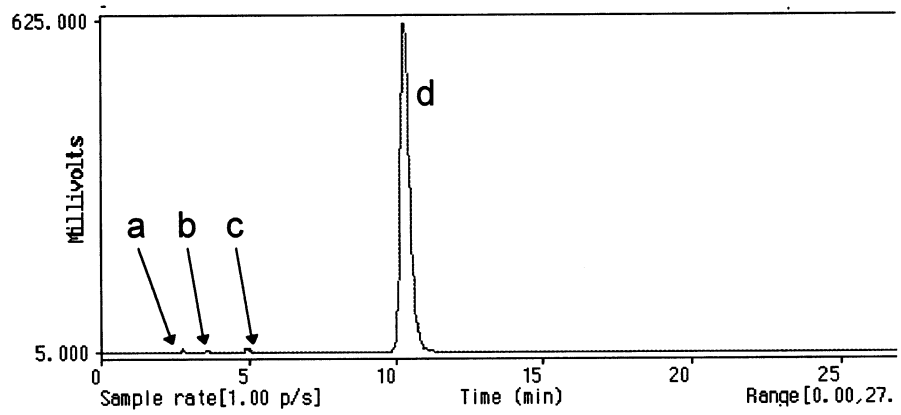

Fig. 2. Chromatogram of the reference standard composite. (a) Degradant 1, (b) compound 1, (c) degradant 2, (d) sunepitron.

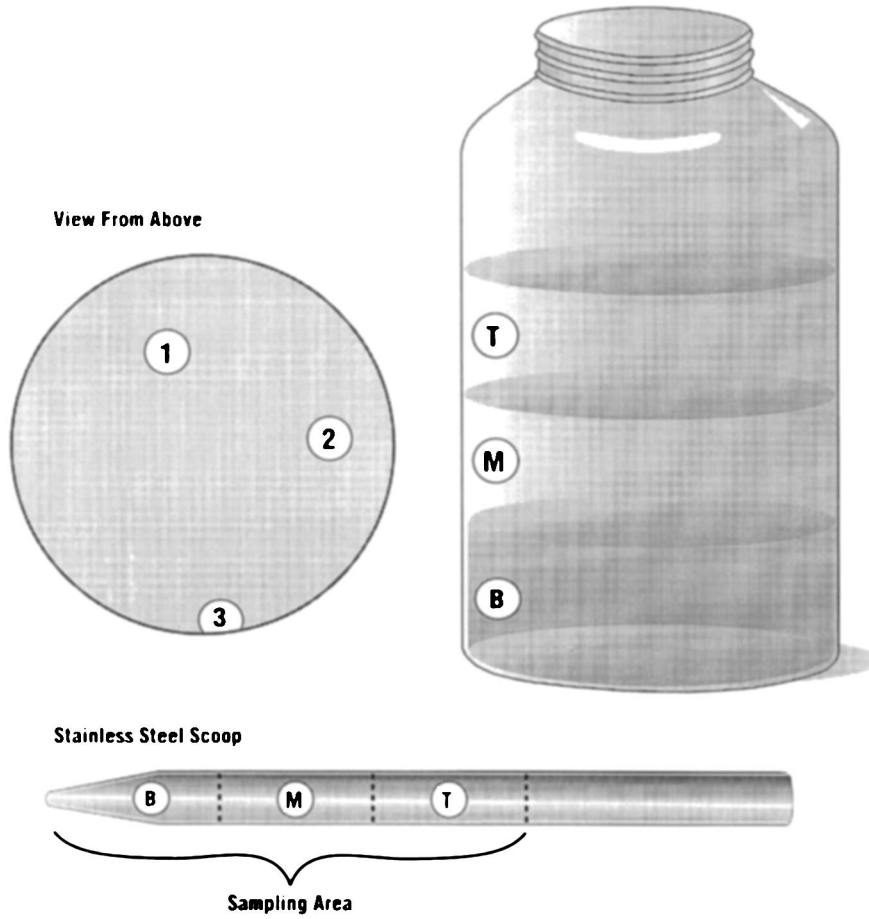

Fig. 3. Sampling diagram for the reference standard composite. T, top; M, middle; B, bottom. 1, 2, And 3 correspond to the regions in each zone where the samples were obtained.

2.2. Preparation of the reference standard composite

The free base of sunepitron hydrochloride was prepared by dissolving the salt in water and adjusting the pH of the solution to 12 with 50% NaOH. The free base (sunepitron) precipitated, was collected by filtration and dried until the water content (Karl Fischer titration) was $<$ 0.1%. The free base was then dissolved in refluxing dichloromethane and 0.5% degradant 1, 0.2% compound 1, and 0.2% degradant 2 (percentages relative to sunepitron) were added to the resulting solution. The mixture was stripped to dryness under vacuum. Analytical results: water content (Karl Fischer), 0.07%; loss on drying (3 h, 60°C, vacuum), 0.09%; residue on ignition (ash), $<$ 0.2%; residual solvents (GC), 0.3% CH_2Cl_2. Additional characterization data is provided in Section 3.

2.3. Instrumentation

The HPLC system used consisted of a Waters (Milford, MA) model 717 autoinjector, a Waters model 510 pump, a Waters model 486 absorbance detector, and a BAS (W. Lafayette, IN) model LC-22A column temperature controller. Waters Puresil™ C_{18} columns, 5 μm particles, 150 mm $\times$ 4.6 mm (part no. WAT044345), were used for all

Table 2
Subsequent assay data for the reference standard composite

Sample	Sunepitron (%)	Degradant 1 (%)	Compound 1 (%)	Degradant 2 (%)
Date of analysis 9/13/96				
11	99.3	0.19	0.13	0.78
12	98.6	0.19	0.13	0.75
13	99.7	0.19	0.12	0.77
14	103.0	0.18	0.13	0.78
15	98.6	0.17	0.14	0.76
16	99.2	0.23	0.14	0.74
17	99.5	0.20	0.13	0.76
18	99.9	0.20	0.12	0.77
19	100.0	0.20	0.13	0.74
20	99.9	0.19	0.11	0.75
Average	99.8	0.19	0.13	0.76
R.S.D. (%)	1.2	8.1	7.2	2.0
Date of analysis 1/8/97				
21	98.0	0.21	0.13	0.79
22	98.1	0.21	0.14	0.81
23	98.0	0.21	0.13	0.81
24	97.9	0.21	0.12	0.78
25	97.1	0.20	0.13	0.79
26	98.7	0.20	0.12	0.81
27	98.8	0.20	0.13	0.82
28	99.4	0.20	0.14	0.83
29	98.7	0.20	0.13	0.83
30	98.4	0.20	0.13	0.82
Average	98.3	0.20	0.13	0.81
R.S.D. (%)	0.6	2.5	5.1	2.1
Date of analysis 3/19/97				
31	97.7	0.20	0.12	0.76
32	97.9	0.20	0.13	0.76
33	97.8	0.20	0.13	0.76
34	97.9	0.20	0.12	0.75
35	98.1	0.20	0.13	0.77
36	98.0	0.20	0.14	0.78
37	98.0	0.20	0.12	0.76
38	98.5	0.20	0.12	0.76
39	98.0	0.20	0.13	0.78
40	98.1	0.20	0.13	0.78
Average	98.0	0.20	0.13	0.77
R.S.D. (%)	0.2	0	5.1	1.4
Total average	98.7	0.20	0.13	0.78
Total R.S.D. (%)	1.1	5.1	5.8	3.4

separations. A Brownlee (Foster City, CA) NewGuard™ scrubber column (part no. 140-601), containing a Brownlee NewGuard™ C18 insert (part no. G18-013), was placed between the pump and the injector. Scrubber columns have increased the lifetime of chromatographic columns developed for other pharmaceutical products and are routinely used as a preventive measure [7]. The 6.5 in. stainless steel lab scoop (cat. no. 57952-162) that was used to sample the reference standard

Table 3
Area percent data for the reference standard composite

Day	Sunepitron	Degradant 1	Compound 1	Degradant 2
3/1/96	98.72	0.27	0.28	0.74
9/13/96	98.65	0.30	0.28	0.77
1/8/97	98.75	0.26	0.27	0.72
3/19/97	98.78	0.26	0.26	0.70

composite was obtained from VWR Scientific, (Baltimore, MD). Moisture microbalance step isotherms were obtained using a VTI (Hialeah, FL) model MB-300 G.

2.4. Chromatographic conditions:

Mobile phase: acetonitrile–MeOH–buffer 6:3:91 (v/v/v) (buffer: aqueous 0.05 M ammonium acetate, pH 4.6); flow rate: 2 ml min^{-1}; detection, 238 nm; injection volume: 10 μl (for the active drug substance) or 100 μl (for the formulated drug product); temperature, 30°C; sample concentration, 1 mg ml^{-1} (drug substance) or 0.1 mg ml^{-1} (drug product).

3. Results and discussion

Reference standard composites must meet two requirements before they can be used as analytical standards for quantitative applications. Each component must be uniformly distributed throughout the sample, and the level of each component must remain constant over time. If degradants are components of the reference standard composite, the storage conditions of the reference standard must minimize further degradation of the active drug substance.

Table 1 illustrates the levels initially determined for sunepitron and each potential impurity. The chromatographic separation is illustrated in Fig. 2. For this analysis, the reference standard composite served as the sample; each component was quantified using external standards. The linearity and recovery of each component from a mixed standard solution has been validated and reported [1]. Each sample was taken from a different location of the bottle holding the spiked reference sample. A stainless steel scoop that mimics an open-face thief used to sample formulated pharmaceutical blends was used for sampling of the spiked reference sample. This sampling approach was used to minimize disruption of the sample and to prevent potential de-mixing of the sample. A sampling diagram is shown in Fig. 3. Sample de-mixing is more prevalent when sampling blends of drug and excipients containing components that differ in physical characteristics such as particle size [8,9]. The sampling approach used here was done as a precautionary measure and may not be necessary for samples of uniform particle size.

A one-way ANOVA analysis of the data indicated that there were no statistical differences in the mean assay values between samples from the top (samples 1–3), middle (samples 4–7), or bottom (samples 8–10) of the bottle. This demonstration of content uniformity throughout the sample bottle indicates that consistent quantitative results would be achieved as the reference standard composite is consumed and as the location in the bottle from where the standard is removed changes.

Subsequent analyses of the reference standard composite are shown in Table 2. The last data points were obtained after storage for more than one year. As a precautionary measure, the reference standard composite was stored under refrigeration in a tightly sealed amber glass bottle. Although all of the Table 2 data are consistent, the results for compound 1 are not consistent with the initial data (Table 1). Since the external standards used for these assays were weighed in duplicate, the error associated with the initial data was probably a result of a dilution and not a weighing error. To support the argument that the compound 1 content was equivalent on each of the

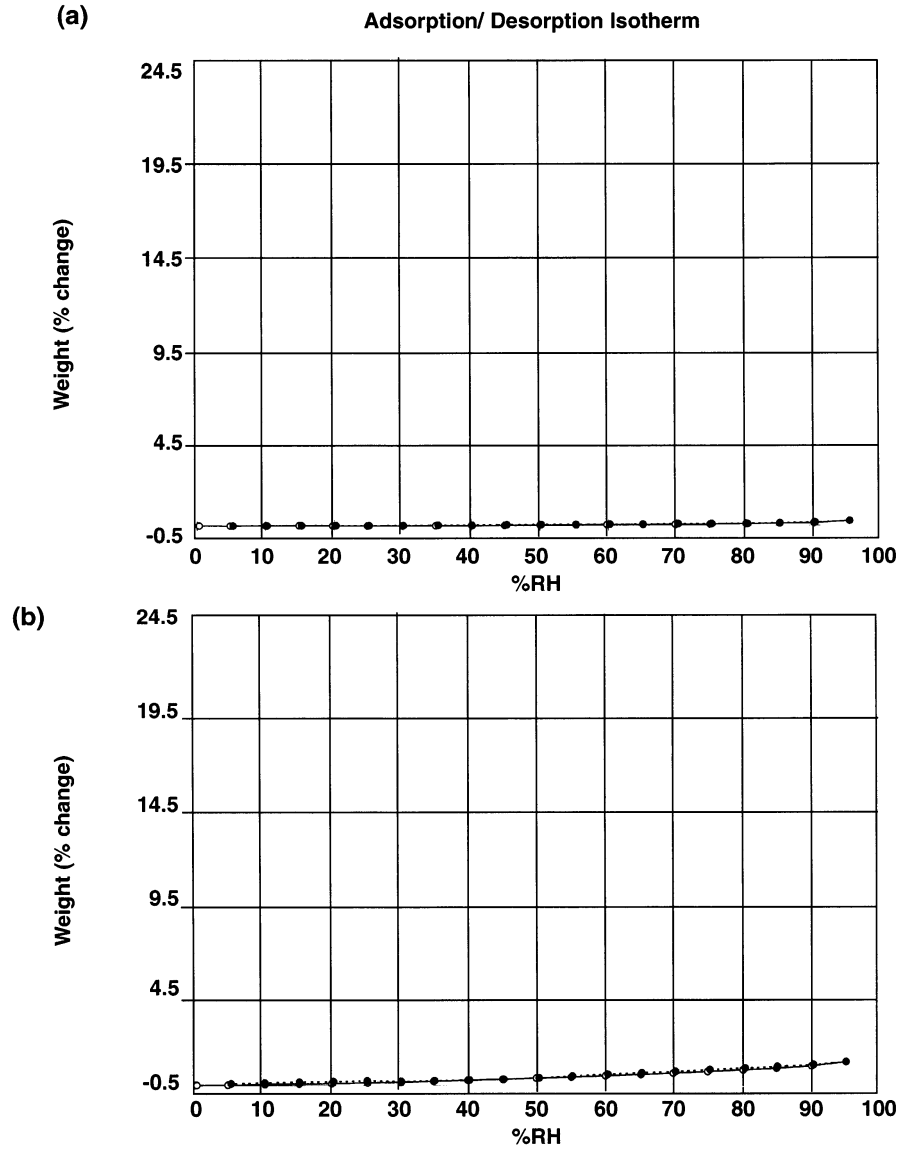

Fig. 4. Moisture microbalance step isotherms for (a) the reference standard composite, (b) sunepitron hydrochloride, (c) compound 1, (d) degradant 1, and (e) degradant 2. ○, Adsorption; ●, desorption.

four analysis days, the average area percent values for each component were compared (Table 3). Since the area percent values for each component were consistent, the composition of the sample has not changed.

The laboratory error associated with the initial compound 1 data illustrates another advantage of reference standard composites. Since all components are weighed simultaneously, it is impossible to incorrectly weigh (or dilute) one component. Since the preparation of individual impurity standard solutions usually involves several dilutions, the potential for error increases dramatically. When impurities are weighed as part of a spiked standard and with judicious selection of the amount weighed and the size of the volumetric

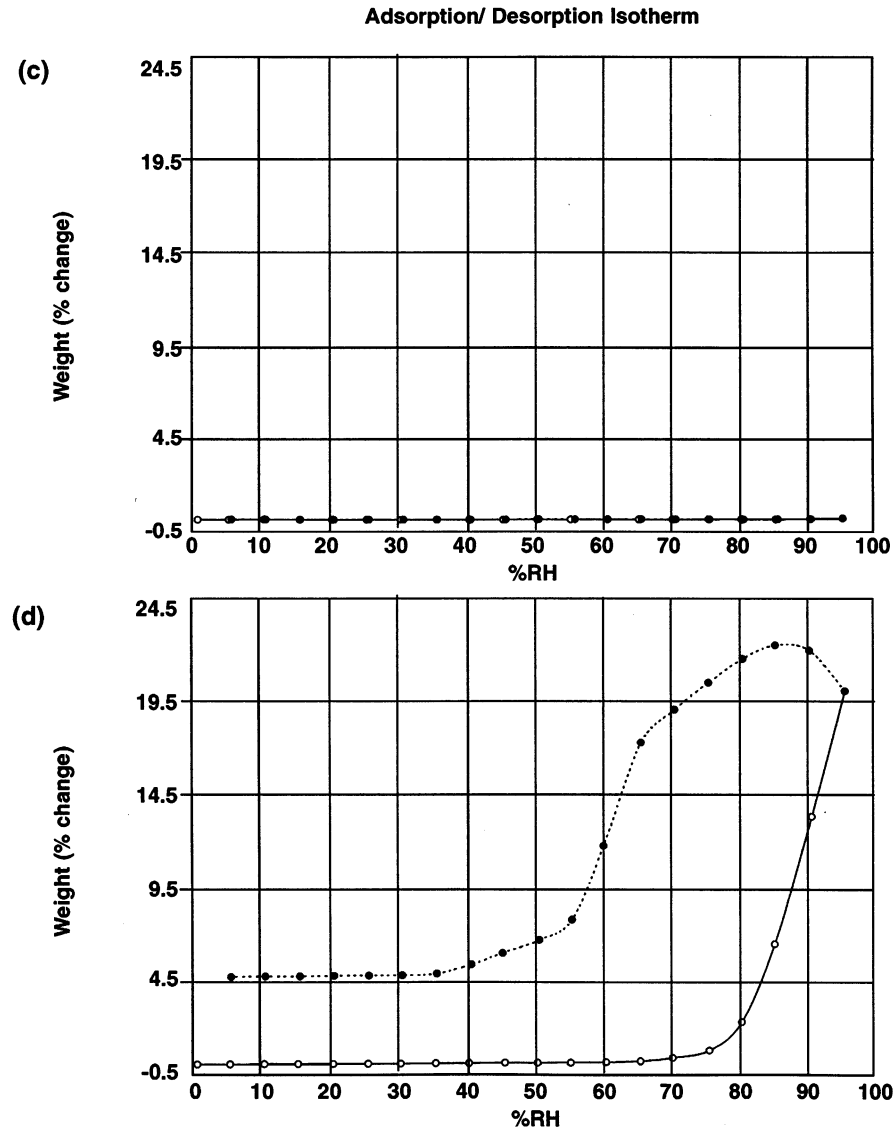

Fig. 4. (*Continued*)

flask, the analytical standard mixture can be transferred into a volumetric flask, brought to volume, and used without further dilutions. Eliminating dilutions also reduces the costs associated with solvents and solvent disposal.

The composite averages of the data generated on three days of analysis in Table 2 were used to assign the sunepitron, compound 1, degradant 1, and degradant 2 levels in the reference standard composite. These are 98.7, 0.13, 0.20, and 0.78%, respectively. The level of compound 1 (0.13%) was lower than the level initially spiked (0.2%). This indicated that this compound was partially purged during the preparation or isolation of the reference standard composite. The level of degradant 1 (0.20%) was consistent with its spiked level (0.2%). The level of degradant 2 (0.78%) was higher than the level spiked (0.5%). This indicated

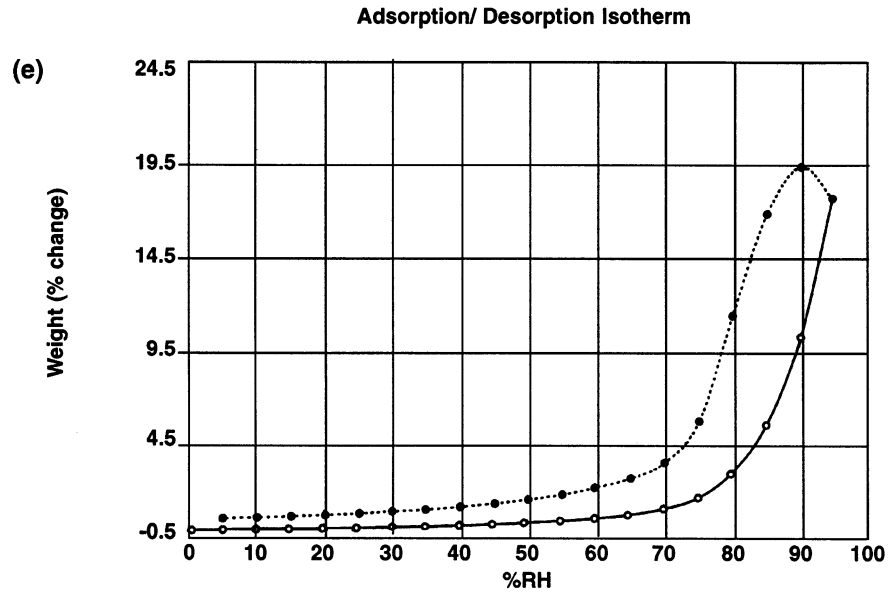

Fig. 4. (*Continued*)

that sunepitron degraded to produce degradant 2 during the preparation or isolation of the reference standard composite. The spiked level of each impurity was chosen based on anticipated target levels. The target level for each impurity is defined as the level at which the quality of the drug substance (or drug product) is questioned. Although the levels of degradant 2 and compound 1 were slightly different from their spiked levels, the resulting reference standard composite is suitable for use as an analytical standard because the levels of each impurity in the reference standard composite were within the levels originally validated for linearity, recovery, and reproducibility [1,2].

A potential advantage of a spiked impurity standard is that the handling properties of the spiked standard may be an improvement over the handling properties of individual standards. Handling properties that could be improved include hygroscopicity, static charge, adhesion, and reactivity. Of the four compounds present in the sunepitron reference standard composite, degradant 1 and degradant 2 are hygroscopic. Moisture microbalance step isotherms for the reference standard composite, sunepitron hydrochloride, and the three potential sunepitron impurities are shown in Fig. 4. The reference standard composite which is not hygroscopic represents a standard with a significant improvement in handling characteristics over standards of the individual impurities.

Although the most beneficial use of reference standard composites is for quantitative assays and purity evaluations, these standards can also be used for qualitative applications such as evaluating system suitability and as retention time markers for process related impurities or degradants. Although each impurity of interest must be present in the injected aliquot, for qualitative applications, the criteria for acceptable content uniformity and stability are relaxed. System suitability for chromatographic assays usually require a test for resolution. The system suitability requirement for resolution is met if a critical pair is adequately resolved. Unless the test samples or standards contain both components of the critical pair, a synthetic mixture must be made and injected. This requires additional time for sample preparation and chromatographic run times. If a spiked reference sample containing the critical pair is injected as part of the chromatographic

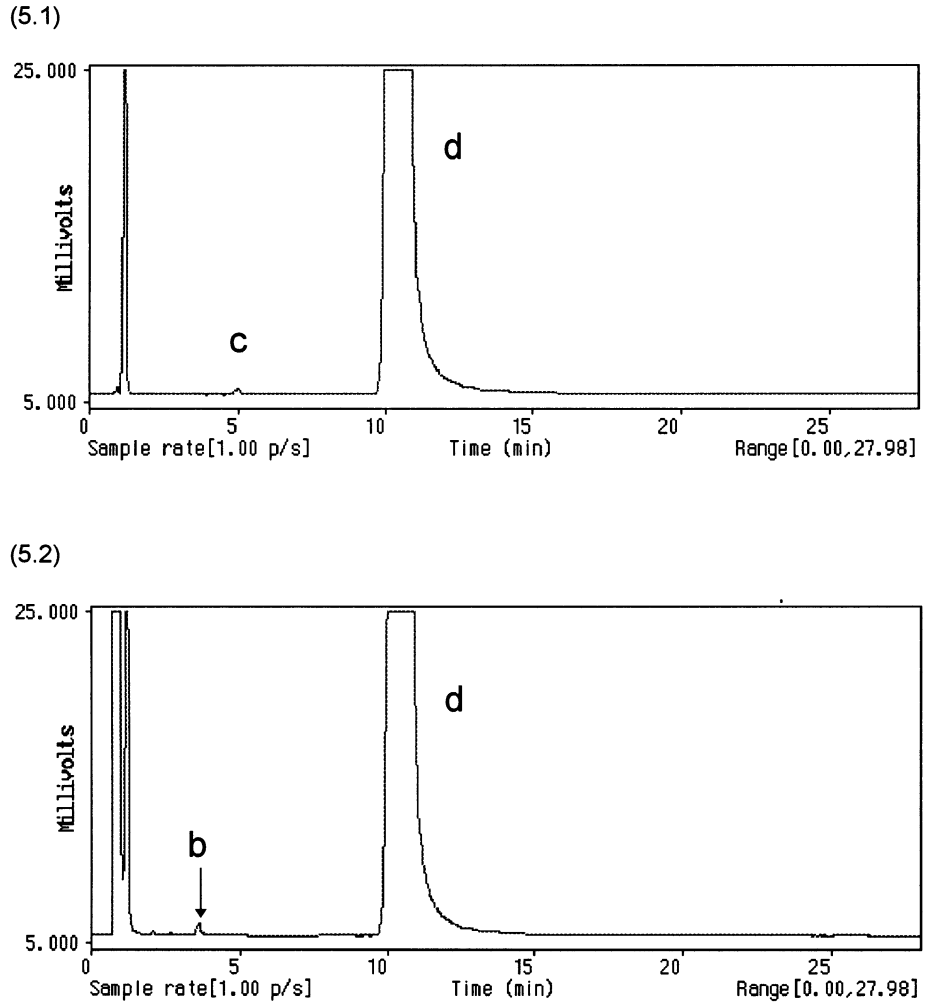

Fig. 5. Chromatograms of (1) sunepitron hydrochloride working standard, (2) sunepitron hydrochloride tablet extract, (3) resolution standard, and (4) the reference standard composite. (a) Degradant 1, (b) compound 1, (c) degradant 2, and (d) sunepitron.

run, additional injections are unnecessary. Shortening chromatographic runs minimizes solvent consumption and the time associated with sample preparation. Fig. 5 illustrates this concept. Assay and purity evaluation of sunepitron hydrochloride tablets using conventional (single component) standards would require injections of the sunepitron hydrochloride working standard (Fig. 5.1), tablet extracts (Fig. 5.2), external standards of the impurities (not shown), and a resolution standard that contains degradant 2 and sunepitron (Fig. 5.3). Since the reference standard composite (Fig. 5.4) contains impurities not present in the sunepitron hydrochloride working standard which are needed for a system suitability resolution test, injection of a separate resolution standard in not necessary.

Another qualitative application for reference standard composites is as a retention time marker for impurities. Although impurity peaks can be tentatively assigned based on their relative retention times, positive identification can only be confirmed after injection of an external standard as part of the same chromatographic run. Since virtually all chromatographic runs are automated and shut down after the last injection, unless

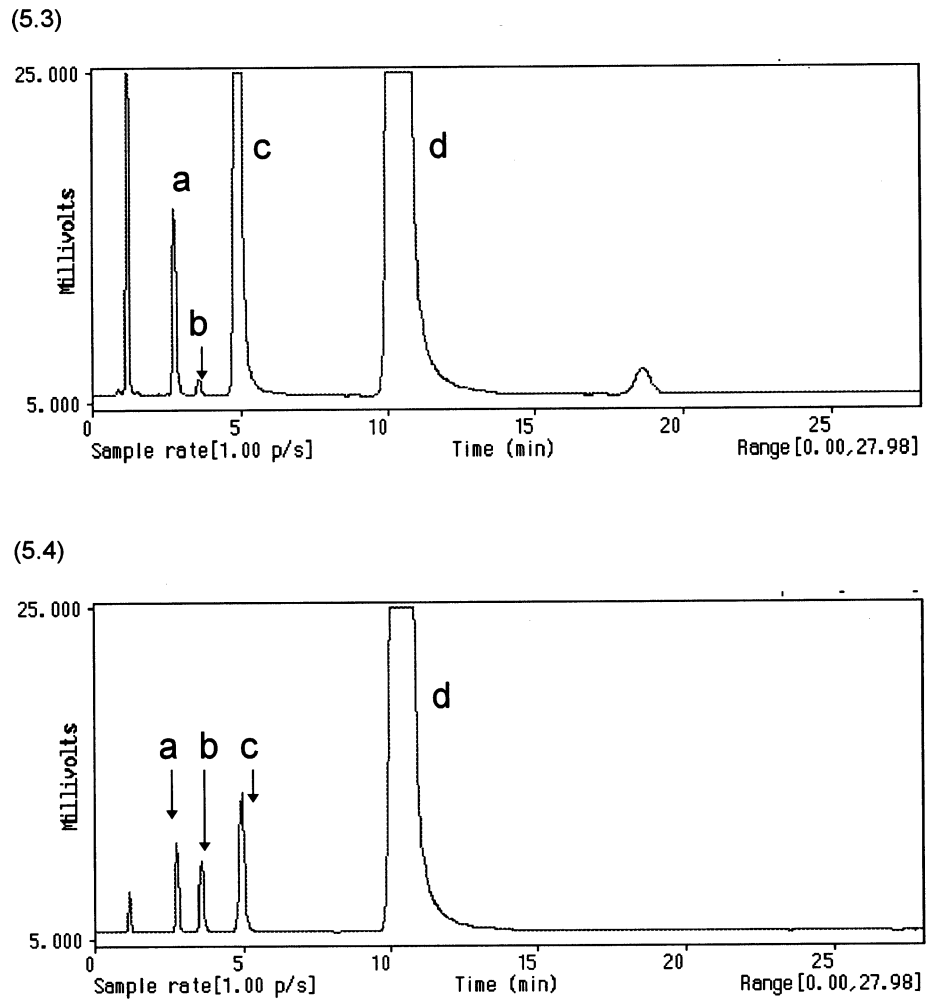

Fig. 5. (*Continued*)

external impurity standards are injected as part of the chromatographic run, it may be necessary to repeat the run with additional injections of impurity standards. For drug candidates in the early stages of development, the impurity profile of samples is often unknown and it may not be practical to prepare and inject external standards of potential impurities which often are available only in limited quantities. Reference standard composites, which minimize the consumption of individual impurity standards provide an authentic standard for several impurities and minimizes their consumption.

4. Conclusions

Reference standard composites contain the active drug substance and potential impurities. These standards have both quantitative and qualitative applications. The feasibility of a reference standard composite containing sunepitron and three potential impurities has been demonstrated. Use of this type of standard saves time in characterization of standards, minimizes the amount of standards consumed, minimizes preparation of standard solutions, and reduces the use of the mobile phase because fewer injections are required.

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