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Investigation of response factor ruggedness for the determination of drug impurities using high-performance liquid chromatography with ultraviolet detection

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Abstract

Quantification of impurities in drug substances and dosage forms using HPLC assays with UV detection is often done by comparison to a standard of the drug itself. Depending on the spectra of the compounds involved, small changes in wavelength may cause response factors to vary from day to day and instrument to instrument. Lack of assay ruggedness with respect to wavelength can lead to poor reproducibility of results. Response factor ruggedness was investigated for potential impurities in LY297802 tartrate, a potent muscarinic agonist. The UV responses of some impurities at 280 nm, the analytical detection wavelength, differ from that of the parent and change significantly with small shifts in wavelength. The ruggedness of response factors was examined on a single detector and among several different detectors. Results varied significantly among the different detectors. The UV spectra of the impurities could be used to predict the effect of wavelength on ruggedness of response. A wavelength system suitability sample is proposed as a way to overcome variability due to small differences in detector wavelength.

Keywords: Response factors; Detector wavelength ruggedness; LY297802

1. Introduction

Detection and quantification of impurities in drug substances and formulations is an important part of drug development. Impurities known as related substances comprise a significant class of impurities that arise from the manufacturing process or via degradation and are structurally related to the drug substance. Recently finalized guidelines for drug substances from the International Conference on Harmonization (ICH) give a threshold value of 0.1% for identification and qualification of a related substance impurity [1]. Reproducible results are, therefore, needed for impurities near this threshold value. Determination of degradation products during stability studies also requires reproducible results for the data to be interpretable.

High-performance liquid chromatography (HPLC) with ultraviolet (UV) detection is widely used for the determination of related substance impurities in bulk drugs and formulated products. The most accurate method of quantifying such impurities is to use external standards of the impurities themselves. This is not always practical since an ample supply of compounds that may be difficult to isolate or synthesize is required. Maintenance of impurity stan-

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dards over several years would also require periodic re-evaluation of the compounds to check stability. For many compounds in development or on the market, each with several potential impurities, such a program rapidly becomes impractical.

Quantification of impurities versus the response of the drug itself is a common alternative to the use of impurity standards [1,2]. This can be accomplished by an area percent method when the main component peak is on scale and within a linear range or with a weight percent calculation versus an external standard of the drug substance. A modification of the area percent method, described by Inman and Tenbarge as the hi/lo method, employs a concentrated sample for high-sensitivity detection of impurities with a dilution of the sample for determining the area of the main peak [3]. Relative response factors are sometimes utilized with these methods to correct for differences in absorptivity between individual impurities and the drug substance.

Reproducibility of results from quantification versus the drug substance can be affected by detector wavelength reproducibility [4–6]. Depending on the spectra of the compounds involved, detector wavelength accuracy and repeatability from day to day and from instrument to instrument may dramatically affect the responses of impurities compared to the drug substance. Accurate conclusions from stability studies can be affected by poor reproducibility of impurity results. Comparison of results between laboratories or establishing whether an impurity is at the ICH threshold can also be confounded by lack of detector wavelength ruggedness.

In this paper we examine the effect of detector wavelength ruggedness for the determination of related substances in LY297802 tartrate, a muscarinic agonist. Findings from this compound are generalized to provide guidance for situations in which wavelength ruggedness could present a reproducibility problem. Also, potential system suitability criteria to reduce variability are described.

2. Experimental

2.1. Reagents

HPLC-grade acetonitrile was obtained from EM Science (Gibbstown, NJ, USA). Trifluoroacetic acid, 99%, was from Aldrich (Milwaukee, WI, USA). Water for mobile phases and sample solutions was purified with a Milli-Q system from Millipore (Milford, MA, USA). Erbium perchlorate ($Er(ClO_4)_3-6H_2O$) was obtained as a 50% aqueous solution from GFS Chemicals (Columbus, OH, USA). 4,4′-dimethoxybenzophenone (97%) and 4-bromobenzaldehyde (99%) were from Aldrich. Samples of LY297802 tartrate and the chloro, ethoxy and hydroxy impurities (Fig. 1) were from Lilly Research Laboratories.

2.2. Apparatus

The chromatographic system consisted of a Model 600 pump (Waters, Bedford, MA, USA), a Model 728 autoinjector (Alcott, Norcross, GA, USA) with a fixed-loop injection valve (Valco, Houston, TX, USA), and several models (see Table 2) of variable-wavelength UV detectors (Applied Biosystems, Ramsey, NJ, USA). Some chromatograms were obtained using a Hitachi Model L-6200A pump (Naperville, IL, USA). Chromatograms were recorded using an in-house data-acquisition system. A 250 mm×4.6 mm I.D. YMC-basic (octyl and lower chain length bonded phase) column with 5 micron particles was used. Ultraviolet spectra were obtained with a Beckman DU 7400 spectrophotometer (Fullerton, CA, USA).

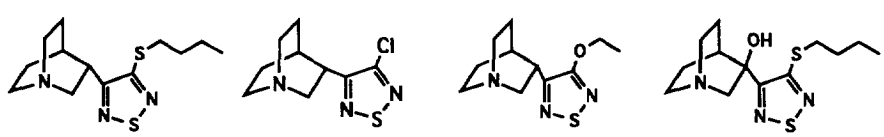

Fig. 1. Structures of LY297802 and impurities.

2.3. Chromatographic conditions

The mobile phase consisted of 65:35 water–acetonitrile containing 0.05% trifluoroacetic acid. The flow-rate was 1.0 ml/min. The injection volume was 20 μl. Samples were dissolved in mobile phase at concentrations in the range 0.02–0.1 mg/ml. The nominal detection wavelength was 280 nm.

3. Results and discussion

The test system used for examination of wavelength ruggedness consisted of LY297802 and related compounds that are potential impurities arising from the synthesis of the drug. Structures and UV spectra of the compounds are shown in Figs. 1 and 2, respectively. Substitution on the thiadiazole ring controls the wavelength of maximum absorption of the compounds [7]. The hydroxy impurity contains the butylthioether substituent and has a spectrum that is nearly identical to that of LY297802 with λ_{max} at 303 nm. The maxima for the chloro and ethoxy impurities are at 269 nm and 277 nm, respectively. A wavelength of 280 nm was chosen to allow reasonable detection of the chloro and ethoxy impurities at a single wavelength, while still allowing for quantification versus LY297802. The ratios of peak area for each impurity to the LY297802 peak area were used as responses for the studies described below. The chloro impurity represents what might be considered a 'worst case' example, since its spectrum is sloping in the opposite direction of the 'standard' and the effect of minor changes in wavelength on the relative response will be magnified. The hydroxy impurity should be a 'best case' situation in that its spectrum is almost identical to that of LY297802, so wavelength changes will affect the response of both compounds similarly.

Reproducibility of response for the same sample solution was determined for multiple injections on one detector (Applied Biosystems Model 757) without changing the wavelength between injections. The ratio of response of each impurity to that of

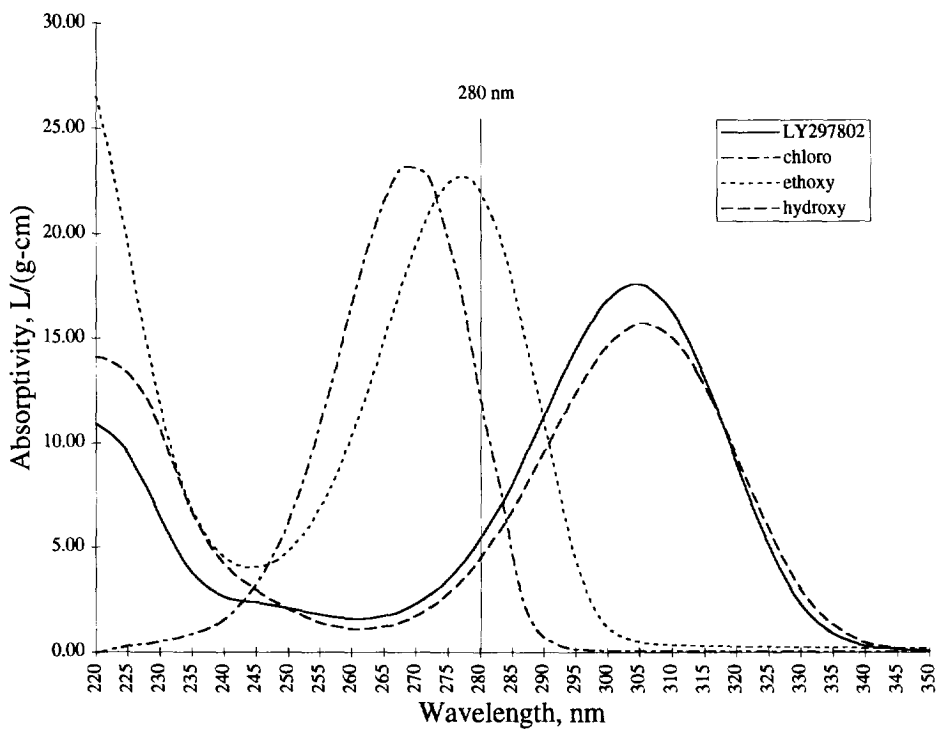

Fig. 2. Ultraviolet spectra of LY297802 and impurities. Sample solvent: water–acetonitrile (65:35) containing 0.05% TFA.

LY297802 was very reproducible (R.S.D.≤0.2%) and consistent with the precision for fixed loop injection. Reproducibility was also checked on one detector where the wavelength was changed and returned to 280 nm between each injection. In this case, variability increased by a factor of ten for the chloro and ethoxy impurities, but remained unchanged for the hydroxy impurity.

More variability for the chloro and ethoxy response ratios with wavelength changes is expected based on the spectra of the impurities compared to LY297802. This was confirmed experimentally, as shown in Table 1, which lists normalized response ratios for each impurity over the range 278–282 nm. This is a quick way to determine if wavelength ruggedness will be a problem with a method, especially if impurities are unknown or are not available in pure form.

Results from the above experiments show the potential for significant variability in relative response ratios based on detection wavelength differences. This variability can arise from differences in wavelength calibration and repeatability. Even if all detectors are calibrated accurately, the repeatability of setting the wavelength from instrument-to-instrument and person-to-person can be a concern [5]. This may be of less concern when using self-calibrating detectors, those with electronic wavelength adjustment, or diode-array detectors.

Detector variability over multiple instruments was investigated by collecting data from several detectors for the same sample solution using the identical mobile phase and column (Table 2). All measurements were made on one day with each detector nominally set at 280 nm. As predicted by the component spectra, the chloro impurity response ratio displayed the greatest variability, while the ratio for the hydroxy impurity was nearly constant. The wavelength calibration of detectors 1, 6 and 12 was checked using erbium perchlorate as described by Esquivel [9]. Consistent with the results given in Table 2, detectors 1, 6 and 12 showed biases of +2, 0 and −2 nm, respectively. Differences of 2 nm for some detectors are consistent with previous results showing offsets as high as 4.9 nm [9]. The specifications for wavelength accuracy and repeatability for all detectors used were ±1 nm and ±0.5 nm, respectively. The calibration results showing 2 nm differences may have resulted from a combination of wavelength bias and repeatability and indicate the difficulty in relying on detector specifications to achieve wavelength ruggedness.

For very low levels of impurities, detector wavelength variability may have no practical significance, but for levels near the ICH qualification/identification threshold of 0.1%, reproducible quantification is critical. Data in Table 2 show that depending on the compound, results could halve or double just by using a different detector.

Table 1
Normalized response ratios versus wavelength

Wavelength (nm)	Impurity		
	Chloro	Ethoxy	Hydroxy
278.0	1.273	1.130	1.000
278.5	1.218	1.106	1.001
279.0	1.151	1.076	1.005
279.5	1.056	1.025	0.995
280.0	1.000	1.000	1.000
280.5	0.910	0.953	1.004
281.0	0.851	0.921	1.004
281.5	0.755	0.868	1.004
282.0	0.706	0.838	1.004

Response ratio = peak area impurity/peak area LY297802, values normalized to response ratio at 280 nm. Applied Biosystems Model 757 detector.

Table 2
Response ratios for impurities at 280 nm on different detectors

Detector	Detector model	Impurity		
		Chloro	Ethoxy	Hydroxy
1	785A	0.553	0.742	0.988
2	757	0.788	0.911	1.061
3	759A	0.812	0.892	0.988
4	759A	0.835	0.920	0.988
5	757	0.941	0.967	1.000
6	759A	0.953	0.977	1.000
7	757	1.047	1.023	1.000
8	783	1.071	1.033	1.000
9	759A	1.129	1.061	1.000
10	757	1.165	1.080	1.000
11	759A	1.176	1.089	1.000
12	757	1.353	1.164	0.988

Responses determined for the same sample solution using the same mobile phase and column on the same day. Response ratio = peak area impurity/peak area LY297802, values normalized to median. All detectors were from Applied Biosystems.

3.1. Prediction of poor wavelength ruggedness

Determination of poor method ruggedness related to detector wavelength is necessary during method development. As demonstrated above, this can be done by comparing results for samples containing the impurities of interest over a narrow wavelength range on both sides of the nominal wavelength. Predictions can also be made by examination of the UV spectra of the impurities and the drug substance being used as the standard. For example, the spectra of many compounds can be approximated as linear over a narrow wavelength range. This is done for LY297802 and impurities in Fig. 3 over a range of 278–282 nm. If the nominal detection wavelength (280 nm) is taken as the zero value on the abscissa, the response ratios can be calculated using Eq. (1) and Eq. (2) for detection at the nominal wavelength and 1 nm greater, respectively:

$$\text{at } \lambda = 280 \text{ nm}, R_0 = \frac{B_i}{B_s} \quad (1)$$

$$\text{at } \lambda = 281 \text{ nm}, R_1 = \frac{M_i + B_i}{M_s + B_s} \quad (2)$$

M_s and B_s are the slope and intercept of the standard spectrum and M_i and B_i are the corresponding values for the impurity spectrum. The effect of a 1 nm change in wavelength can be calculated by taking the ratio of the response ratios given above:

$$\text{effect of a 1 nm change} = \frac{R_1}{R_0} = \frac{M_i + B_i}{M_s + B_s} \times \frac{B_s}{B_i} \quad (3)$$

The results calculated for LY297802 impurities using Eq. (3) are as follows: chloro, 0.80; ethoxy, 0.90; hydroxy, 1.00. These are consistent with data in Table 1. Also, examination of Eq. (3) reveals some general conclusions regarding the influence of spectra on response ruggedness with respect to small wavelength changes.

1. If the slopes of the standard and impurity spectra are low, i.e., the spectra are flat over the range of interest, the results will be reproducible.
2. If one of the intercept values is low compared to the other, the results will be less reproducible.
3. Results are more reproducible with higher intercept values.
4. Poorest reproducibility is obtained with low intercepts and high slopes of opposite sign.

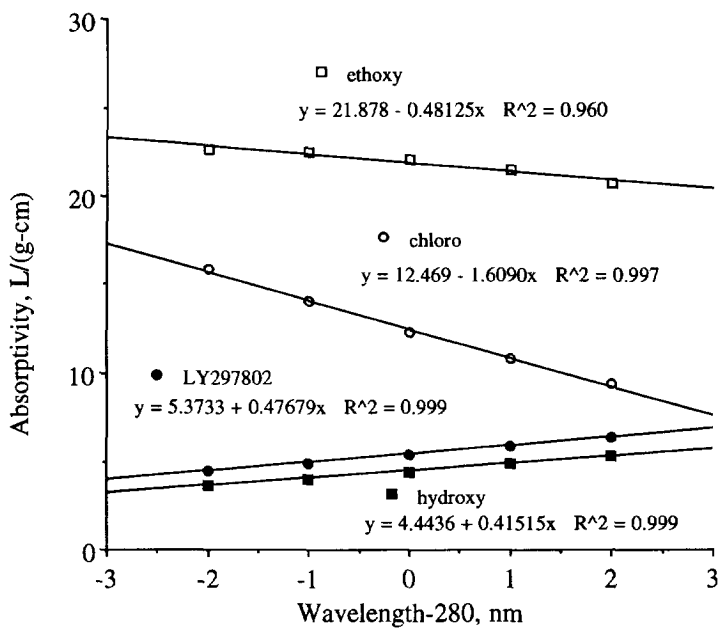

Fig. 3. Linear fit for UV spectra of LY297802 and impurities at 280±2 nm.

These conclusions may be arrived at intuitively, but Eq. (3) provides a useful tool for evaluating the significance of wavelength changes based on the spectra of the compounds in question.

3.2. Detector wavelength system suitability

If quantification must be done versus the parent compound and it is known that impurity response factors are not rugged toward small differences in wavelength, then several steps can be taken to ensure reproducible results. If possible, the method could be run on one dedicated instrument without changing the detector wavelength between runs. This may be feasible over a short time period but cannot be done if implementing the method in different laboratories. Accurate wavelength calibration of all detectors that might be used could help minimize variability [5,8,9], but the frequency of calibration that is needed may be difficult to determine. Suitable calibration at the desired analytical wavelength may be a concern. Also, calibration does not address variability or errors in setting the wavelength from run to run.

Employing a wavelength system suitability sample would ensure reproducible impurity response factors for any combination of instrument or analyst. Such a sample would need to contain impurities whose responses can be used to set the appropriate wavelength. An example is shown in Fig. 4 for LY297802 tartrate that contains the hydroxy, chloro and ethoxy impurities. In this sample, the ratios of chloro and ethoxy peak areas to that of the hydroxy impurity (spectrum similar to that of LY297802) are sensitive to small wavelength changes. If the method specified that a given ratio for the two peak areas must be obtained, the wavelength could be adjusted until that ratio is achieved. This approach requires that an ample supply of system suitability sample is available and that the impurity levels in the sample are stable.

A similar approach is to use stable commercially available compounds whose responses relative to each other vary significantly around the wavelength

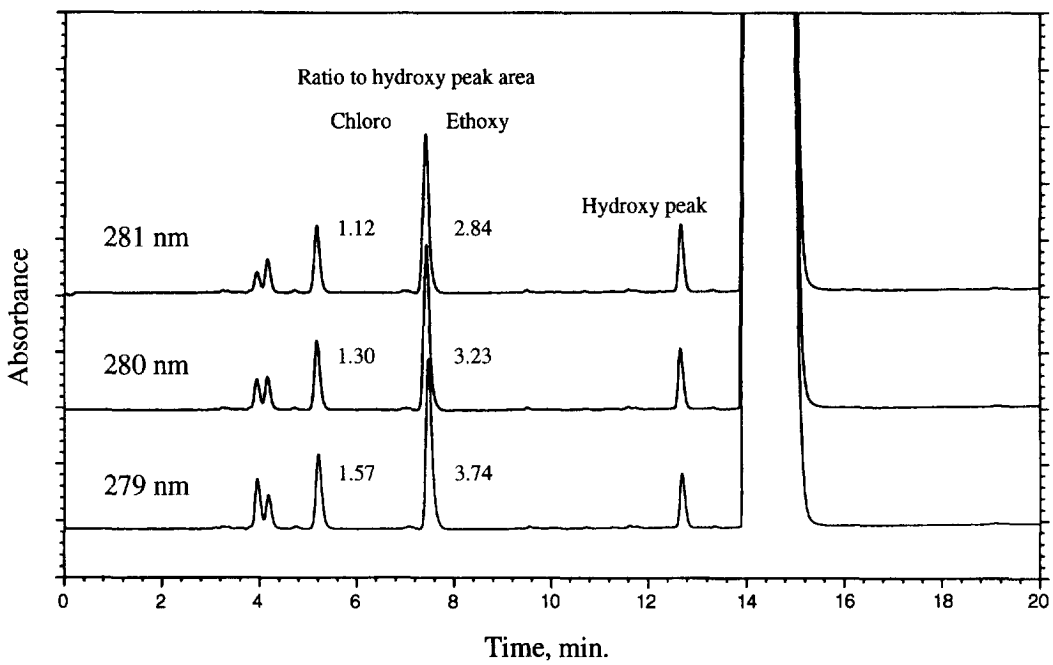

Fig. 4. Wavelength system suitability sample for reproducible determination of impurities in LY297802. Column: 25 cm×4.6 mm YMC basic, 5 micron particles. Mobile phase components: A=0.05% trifluoroacetic acid in water, B=0.05% trifluoroacetic acid in acetonitrile. Gradient program: 25% B to 60% B in 15 min, hold at 60% B for 10 min. Flow-rate: 1.0 ml/min. Injection volume: 20 μl. LY297802 tartrate concentration: 4 mg/ml in initial mobile phase.

Table 3
Response ratios for 4,4′-dimethoxybenzophenone (DMBP) and 4-bromobenzaldehye (BBA)

Wavelength (nm)	Area BBA/area DMBP
238.0	0.59
239.0	0.70
240.0	0.83
241.0	0.99
242.0	1.21
278.0	1.27
279.0	1.10
280.0	1.00
281.0	0.89
282.0	0.80

Column: Zorbax RX-C8, 250 mm×4.6 mm I.D., 5 micron particle size. Mobile phase: 45%–0.05% trifluoroacetic acid in water, 55%–0.05% trifluoroacetic acid in acetonitrile. Flow-rate: 1.0 ml/min. Injection volume: 20 μl. Sample solvent: mobile phase. Sample concentration: for 280 nm evaluation, DMBP=21 μg/ml, BBA=56 μg/ml; for 240 nm evaluation, DMBP=42 μg/ml, BBA=22 μg/ml.

of interest. Data using this approach are given in Table 3 for 4,4′-dimethoxybenzophenone and 4-bromobenzaldehyde. The spectra of these compounds slope in opposite directions at 280 nm and provide a sensitive way to adjust the detector to a predetermined peak area ratio for a specified concentration of each component. These compounds are separated in less than 10 min using a mobile phase differing only in solvent strength from that employed in the analysis of LY297802 tartrate. This mixture would provide a quick convenient wavelength system suitability sample to ensure consistent response factors. They could be used for a similar purpose at wavelengths near 240 nm where the spectra are also sloping in opposite directions. Other compounds with appropriate spectral characteristics could be used for other wavelengths.

The need for a wavelength system suitability sample should be carefully considered. The variability that can be accepted for quantification of a given impurity is often dependent on the concentration of the impurity. For example, variability of ±20% may be acceptable for an impurity at a level of 0.02%, but not at 0.09%. An evaluation of the predicted variability using Eq. (3) can be helpful in this regard.

4. Conclusions

Detector wavelength variability from a nominal value can cause significant variability in relative response factors for drug impurities that are quantified versus the drug substance. The magnitude of this variability is a function of the spectra of the impurities and drug substance and can be predicted from the spectra or determined from chromatograms at different wavelengths. In cases where very reproducible results are needed, a wavelength system suitability sample can be employed to ensure that the impurity response factors on any detector match those from previous analyses.

Acknowledgments

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