Determination of relative UV response factors for HPLC by use of a chemiluminescent nitrogen-specific detector

M.A. Nussbaum *, S.W. Baertschi, P.J. Jansen

Chemistry Department, Hillsdale College, 33 E. College St., Hillsdale, MI 49242, USA

Abstract

Ultraviolet (UV) absorbance is the most widely used detection method for high-performance liquid chromatography (HPLC) separations. In pharmaceutical analysis, purity determinations often include quantitation of related impurities based on relative HPLC peak areas obtained at a specific wavelength. In order for this quantitation to accurately reflect weight percentages of impurities, the relative UV response factors (absorptivities) at the given wavelength must be known. In this work, we present a convenient method for determining relative UV response factors on-line, without isolation or purification of impurities, without standards, and without requiring known analyte concentrations. The procedure described makes use of a chemiluminescent nitrogen-specific HPLC detector (CLND) in conjunction with a UV detector. The CLND response is directly proportional to the number of moles of nitrogen in each eluting peak, and can, therefore, be used to determine relative amounts of each nitrogen-containing impurity present in the sample, provided the molecular formulas are known (e.g. from exact mass LC–MS). It is a simple matter, then, to determine the relative UV response factors from the UV area ratios obtained for the same sample. The feasibility and accuracy of this method is demonstrated for gradient HPLC separations of commercially available compounds of widely varying structures. Finally, the method's utility in obtaining accurate mass balance is demonstrated by application to photodegradation of nifedipine. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Response factors; Absorptivity; Chemiluminescence; Nitrogen detection; HPLC detection; Mass balance

1. Introduction

High-performance liquid chromatography with ultraviolet absorbance detection (HPLC-UV) is widely used for separation and quantitation of impurities. In pharmaceutical analysis, the determination of drug purity generally includes quantitation of all detected peaks based on relative peak areas obtained at a specific wavelength. In order for such quantitation to accurately reflect weight percentages of impurities, the relative responses (absorbance per unit weight) at the given wavelength must be similar. If, as is often the case, the response factors differ significantly between impurities and the parent compound, then correction factors need to be applied to the impurity peak areas in order for them to be correlated to weight

* Corresponding author. Tel.: +1-517-607-2612.
E-mail address: mark.nussbaum@hillsdale.edu (M.A. Nussbaum).

percentages. Thus, the determination of relative response factors (RRF) of impurities is integral for assessing purity of a given sample by HPLC-UV. In this paper we describe a novel and convenient way to accurately determine such relative UV response factors.

Traditionally, the process for establishing response factors involves the use of 'standards', or isolated samples of individual impurities, from which accurate concentrations can be prepared. The purity of each such sample must, therefore, be known. This is a relatively straightforward procedure if such impurities can be readily synthesized and recrystallized. The purity of these synthetically-prepared samples is generally estimated using a combination of HPLC (with UV, light-scattering, or other appropriate detection), nuclear magnetic resonance (NMR), and some method to determine volatile impurities (e.g. TGA, Karl Fischer). The process often requires assumptions about the identity and levels of contaminants that may be present, and involves a significant amount of time and effort (i.e. expense). The effort required is typically even greater for impurities for which synthetic samples are not readily available (e.g. low-level process impurities and degradation products). Such impurities need to be isolated and purified using standard techniques such as preparative TLC or HPLC. The risk of having adventitious contaminants in the isolated impurities is greater than for synthetic samples because of the large amounts of solvents used, the possibility of non-chromophoric contaminants (e.g. solvents or column bleed), the presence of counter-ions (e.g. trifluoroacetic acid (TFA), acetate, etc.), and the impracticality of using crystallization (because of the low amounts isolated) to enhance the purity. Moreover, in many cases the impurities are unstable and thus very difficult to purify. In order to determine sample purity, amounts of 50 mg or more are typically needed. Isolation of these amounts of impurities can be very time-consuming and costly. Thus, a simpler method for determining response factors that avoids the need to isolate and characterize impurity samples would save considerable time and effort.

A potential alternative for determining UV response factors would use two HPLC detectors: a standard UV absorbance detector, and a detector that has a response proportional to weight. For example, if a second detector could provide accurate information on the relative amounts of the impurities and parent compound in a sample mixture, then this information, combined with the UV peak areas, would supply the desired RRF information without the need for a purified impurity sample. Unfortunately, most HPLC detectors do not provide accurate quantitative information unless a standard of the given analyte is available for comparison. Some 'universal' HPLC detectors will give a response for most compounds, but that response is not uniformly related to weight. For example, evaporative light scattering (ELSD) allows detection of most non-volatile substances, but the detector response can be highly variable because it depends on the quantity and nature of the particles produced upon the desolvation process occurring in the detector. Only for compounds of very similar structures can one expect similar responses per unit mass by ELSD, and even then, the variability is 10–20%. For compounds of widely varying structures, charges, or vapor pressure; or for varying mobile phase compositions (e.g. gradient HPLC), the ELSD response can vary markedly [1–7]. Similarly, mass spectral (MS) detectors are universal, but the response per unit weight depends greatly on the ionization type (e.g. electrospray, atmospheric-pressure chemical ionization, etc.) and on the ionization efficiency of the analyte under the given conditions. Refractive index is another universal detector, but it too suffers from variability in response depending on the mobile phase composition, temperature, and dissolved gases; furthermore, it is relatively insensitive [3,8].

Recently, a chemiluminescent nitrogen-specific HPLC detector (CLND) has become commercially available. This detector is based on combustion of the HPLC effluent in an oxygen-rich furnace to convert all organic species to oxides of carbon, nitrogen, sulfur, etc. and water. The nitric oxide produced from nitrogen-containing compounds is then reacted with ozone to produce nitrogen dioxide in an excited state, which emits photons upon return to the ground state (see Fig. 1 for a sche-

matic of the instrument). This chemiluminescent response is proportional to the number of moles of nitric oxide, and correspondingly to the number of moles of nitrogen originally present in the analyte. For virtually any nitrogen-containing compound (with the exception of N_2 and compounds containing N=N bonds), the signal is independent of structure. Amines, amides, nitrates, nitrogen-containing heterocycles, etc. all produce a signal that is directly related to the number of moles of nitrogen present. Provided the molecular formula of the analyte is known, one can thus determine its relative weight in the sample from the amount of nitrogen in the HPLC peak. Quantitation, then, requires only a single nitrogen-containing standard, which need not be structurally related to the analyte. Thus, the CLND provides a unique and demonstrated capability for determining sample concentrations without the need for standards of each analyte [9,10].

Moreover, for determination of *relative* amounts, no standard whatsoever is necessary. All that are needed are the relative CLND peak areas and the molecular formulas of the analytes. Once the relative amounts are found, it is a simple matter to use UV peak areas (e.g. from a UV detector in series with the CLND) to determine the RRF. Thus, UV response factors (per unit weight) for impurities, relative to parent compounds, can be determined by means of the following equation

$$RRF_\lambda = \frac{[(UV\ peak\ area)/(CLND\ peak\ area)]_i}{[(UV\ peak\ area)/(CLND\ peak\ area)]_p}$$
$$\times \frac{(M.W./\#\ nitrogen)_p}{(M.W./\#\ nitrogen)_i} \qquad (1)$$

where i is impurity; p denotes parent compound; M.W. is molecular weight; # nitrogen is the number of nitrogens in the molecular formula.

For unknown impurities, exact mass LC–MS can be used to determine the molecular formula [11–13]. For molar (rather than weight) RRF values, one needs only the relative number of nitrogens per molecule and not the molecular formula or weight. By use of the CLND, then, relative UV response factors can be determined without fraction collection or purification, without standards, and even without preparations of known concentrations of analytes. As a result, sample preparation is greatly simplified, and the stability of the impurity is not an issue.

The CLND is limited, of course, to mobile phases that do not contain nitrogen. Acetonitrile and amine modifiers, commonly used in HPLC, are, therefore, precluded. Also, the CLND is not readily amenable to non-volatile buffers in the
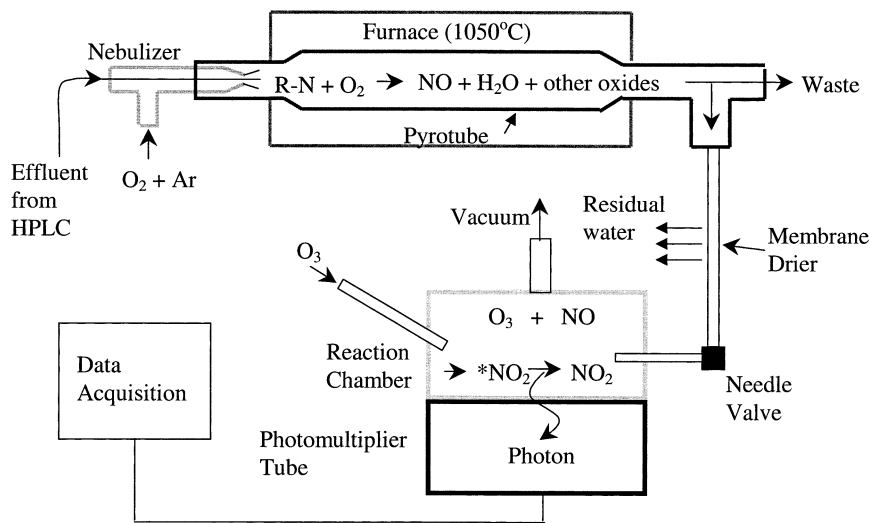

Fig. 1. Schematic of CLND instrumentation.

mobile phase. However, it is still possible to determine relative UV response factors for samples run under these non-CLND-compatible HPLC conditions. One option is to analyze a sample with the CLND first (using a compatible mobile phase) to determine the relative amounts of the analytes present. Then the *same sample* is analyzed with UV detection using non-CLND-compatible HPLC conditions, and the RRF calculated using Eq. (1). Such an approach assumes that the sample is unchanged (i.e. no degradation or change in analyte concentration) between the two analyses. Often, especially when multiple laboratories and conditions are involved, it may be preferable to use a second approach. In this case, a CLND-compatible mobile phase is used to separate the compounds of interest and determine RRF values under the given conditions (RRF_1). Separately (e.g. at a laboratory without access to the CLND) and as needed, a sample is assayed by HPLC-UV using both the original CLND-compatible mobile phase and the non-compatible mobile phase of interest. The relative UV peak areas are then used to correct the RRF value for the change in conditions. Thus, RRF_1 obtained originally with the CLND-compatible conditions can be used to determine RRF_2 for any different set of conditions by multiplying by the ratio of the relative UV areas obtained under each.

$$RRF_2 = RRF_1$$

$$\times \frac{(UV \ peak \ area)_{i,2}/(UV \ peak \ area)_{i,1}}{(UV \ peak \ area)_{p,2}/(UV \ peak \ area)_{p,1}}$$

$$(2)$$

where i is impurity; p denotes parent compound; and 1 and 2 represent CLND-compatible and non-compatible HPLC conditions, respectively.

The approach described with Eq. (2) permits running a separate sample at any time at a site removed from the CLND, albeit that sample must be run with UV detection under both sets of HPLC conditions. In pharmaceutical development, where HPLC conditions may be modified across several sites and over a period of years, such flexibility is quite valuable. The RRF values for key impurities can thus be determined at a core laboratory early in development using a

given set of CLND-compatible conditions. As development progresses and HPLC conditions change, the RRF values can be continuously corrected and updated simply by use of Eq. (2), which requires only an HPLC-UV system and access to the original data.

Finally, the RRF value is independent of whether the given impurity is present at trace levels or at significant amounts. Thus, in pharmaceutical development, forced degradation may often be used to generate relatively large amounts of key impurities, such that both UV and CLND peak areas may be precisely measured. The accurate RRF values thus obtained may then be applied widely to other situations in which the impurities are present at much lower levels.

Previous reports have demonstrated the unique capability of the CLND to accurately measure concentrations of nitrogen-containing compounds without the need for standards of the same or similar compounds [9,10]. In this paper, we demonstrate the application of this attribute to the simple and accurate determination of relative UV response factors for a variety of compound structures, by gradient as well as isocratic HPLC. We also demonstrate how this capability can be important in resolving mass balance issues, which occur frequently during forced degradation studies, using photodegradation of nifedipine as an example. Finally, important considerations and potential pitfalls in using this system are addressed.

2. Experimental

2.1. Equipment

The model 8060 CLND was purchased from Antek Instruments (Houston, TX, USA). For the gradient test-mix experiments, the CLND parameters were as follows, furnace at 1050 °C, oxygen, argon, make-up, and ozone flows set at 180, 150, 30, and 25 cm^3/min, respectively. For the nifedipine work, the CLND conditions were the same except the oxygen, argon and make-up flow settings were 200, 100, and 60 cm^3/min, respectively. These latter settings resulted from
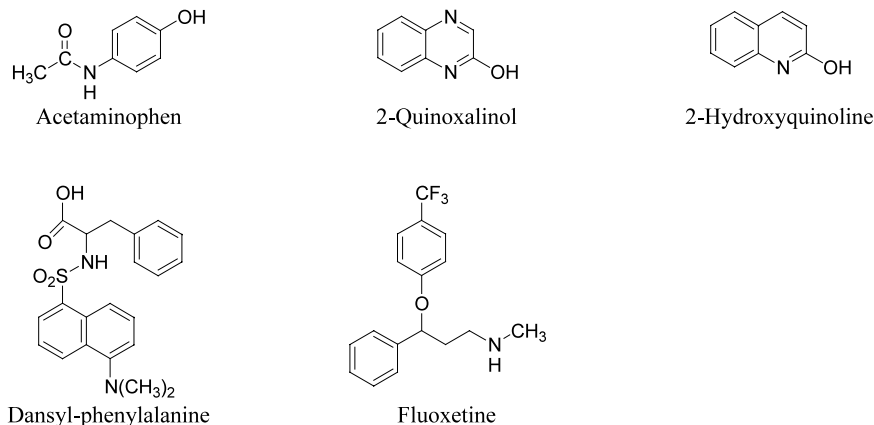
Fig. 2. Structures of test compounds.

greater experience with the detector, and should be suitable for the test-mix experiments as well. The evaporative light-scattering detector used was an Alltech 500 (Alltech Associates, Deerfield, IL, USA), with a drift-tube temperature of 65 °C and a gas flow of 3 l/min. Two HPLC systems were used, (1) a Hewlett–Packard (Agilent Technologies, Wilmington, DE, USA) series 1050 (including pump, autosampler, and UV detector); and (2) a Thermo-Separation Products (ThermoQuest, San Jose, CA, USA) system consisting of a P4000 narrow-bore gradient pump, an AS3000 narrow-bore variable-loop autosampler, and UV1000 single wavelength detector. A Zorbax (Agilent Technologies) Rx-C18 HPLC column, 2.1×150 mm (5 μm particles) was used for the nifedipine mass balance work, and a Zorbax Rx-C8 column of the same dimensions was used for all other separations. Note that narrow-bore columns were used in conjunction with the low liquid flow rates (< 400 μl/min) required by the CLND; it is also possible to use wider bore columns and faster flow rates if a flow-splitter is incorporated. Photodegradation of nifedipine was done using a Fiber-Lite series 180 high-intensity illuminator (Dolan-Jenner Industries, Lawrence, MA, USA). UV spectra of nifedipine and its degradation product were obtained using a Waters (Milford, MA, USA) 996 photodiode array HPLC detector.

2.2. Materials

Acetaminophen, dansyl-phenylalanine (cyclohexylammonium salt), and nifedipine were purchased from Sigma (St. Louis, MO, USA). Phosphoric acid, 2-quinoxalinol, and 2-hydroxyquinoline were obtained from Aldrich (Milwaukee, WI, USA). Fluoxetine–HCl was obtained from Lilly Research Laboratories (Eli Lilly and Company, Indianapolis, IN, USA). TFA was from Pierce Chemical (Rockford, IL, USA), and potassium phosphate monobasic was from EM Science (Gibbstown, NJ, USA). Phosphoric acid and HPLC-grade methanol (MeOH) and acetonitrile (ACN) were obtained from Fisher Scientific (Pittsburgh, PA, USA). HPLC-grade water was from a Milli-Q UV-Plus system (Millipore, Bedford, MA, USA). Phosphate buffer was prepared by dissolving 21.8 g of KH_2PO_4 in 4.0 l of water and adjusting the pH to 3.0 with phosphoric acid, prior to mixing with acetonitrile.

All HPLC mobile phase compositions are listed on a volume percentage basis.

3. Results and discussion

3.1. Accuracy

In order to determine the accuracy of CLND-determined RRF values, several high-purity, com-

mercially available compounds were used: acetaminophen, 2-quinoxalinol, 2-hydroxyquinoline, dansyl-phenylalanine, and fluoxetine. Their structures are shown in Fig. 2. Note that these compounds encompass a wide range of volatility, hydrophobicity, and nitrogen-containing functional groups. All were at 99% or greater purity. Arbitrarily, all response factors are reported relative to fluoxetine.

Accurately weighed quantities of each compound were combined in a test mixture that was separated by gradient HPLC. Both CLND-compatible (TFA/MeOH/H$_2$O) and non-compatible (PO$_4$/ACN/H$_2$O) HPLC separations were performed with UV detection at 230 nm. Peaks were integrated and true RRF values were calculated for each compound by dividing the UV peak area per unit weight by that of fluoxetine. In addition, the CLND was used with the TFA/MeOH/H$_2$O mobile phase, and the RRF values were calculated using Eq. (1), without sample weights. Fig. 3

shows chromatograms obtained with both CLND and UV using the TFA/MeOH/H$_2$O mobile phase. The corresponding RRF values under the HPLC conditions containing acetonitrile and phosphate (not directly compatible with the CLND) were calculated from the relative UV responses as in Eq. (2).

The resulting RRF values are tabulated in Table 1. Note that the RRF values obtained by using the CLND areas are all within 8% of the values obtained using the known weights for each compound. Thus, the CLND provides a reasonably accurate and simple way of obtaining RRF values for these compounds, despite the gradient HPLC conditions and dissimilarities in structure.

By contrast, ELSD gives widely varying responses for these compounds. Fig. 4 shows the UV and ELSD chromatograms obtained for the test compounds (concentrations are given as mg/ml of the free acid or base). The ELSD temperature had to be reduced from 90 to 65 °C in order
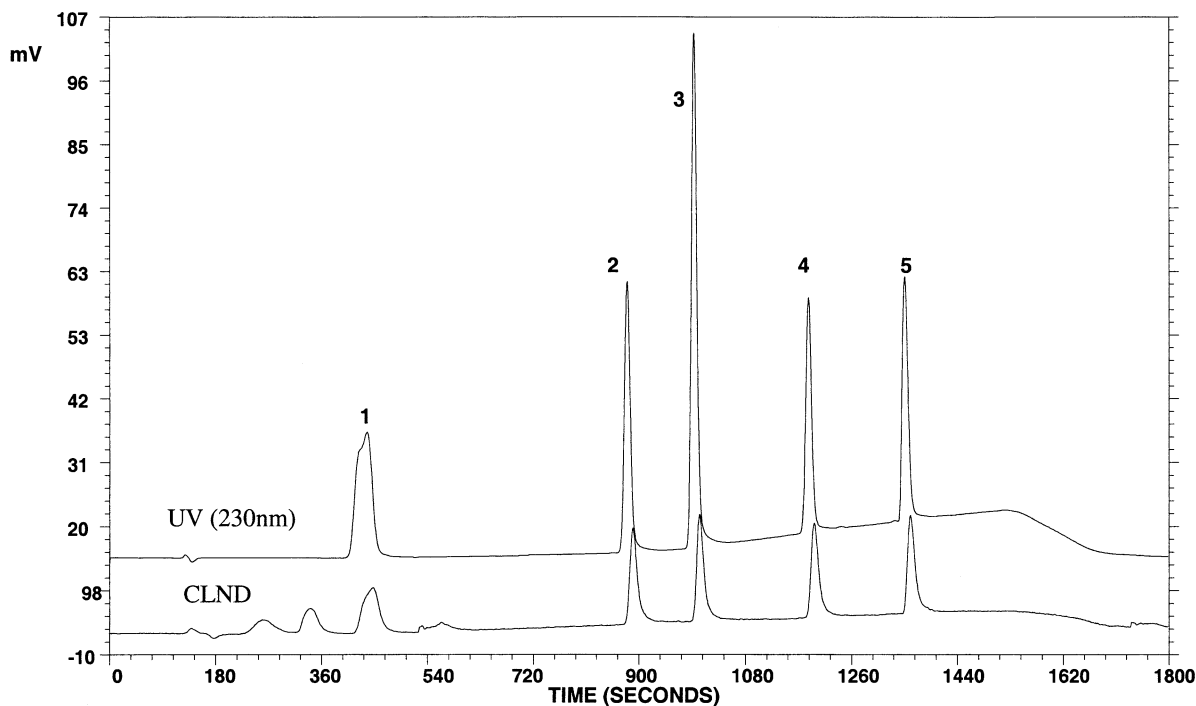

Fig. 3. UV (230 nm) and CLND detection of test mixture (1, acetaminophen; 2, 2-quinoxalinol; 3, 2-hydroxyquinoline; 4, dansyl-phenylalanine; 5, fluoxetine) 2 mM nitrogen each, 8 μl injection; column: Zorbax Rx-C8, 0.21 × 15 cm. Mobile phase: 0.1% TFA in 10–82% MeOH, 0.2 ml/min.

Table 1
RRF at 230 nm as determined from known compound weights or from CLND peak areas

Compound	RRF$_1$ by known weights[a]	RRF$_1$ by CLND peak areas[a]	RRF$_2$ by known weights[b]	RRF$_2$ by CLND peak areas[b]	CLND accuracy (%)
Acetaminophen	2.63	2.83	3.58	3.85	107.6
2-Quinoxalinol	4.64	4.77	6.48	6.66	102.8
2-Hydroxyquinoline	4.15	4.18	5.83	5.87	100.6
Dansyl-Phenylalanine	1.46	1.51	2.14	2.21	103.3
Fluoxetine	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	N/A

[a] HPLC conditions (RRF$_1$): methanol/water/0.1% TFA.
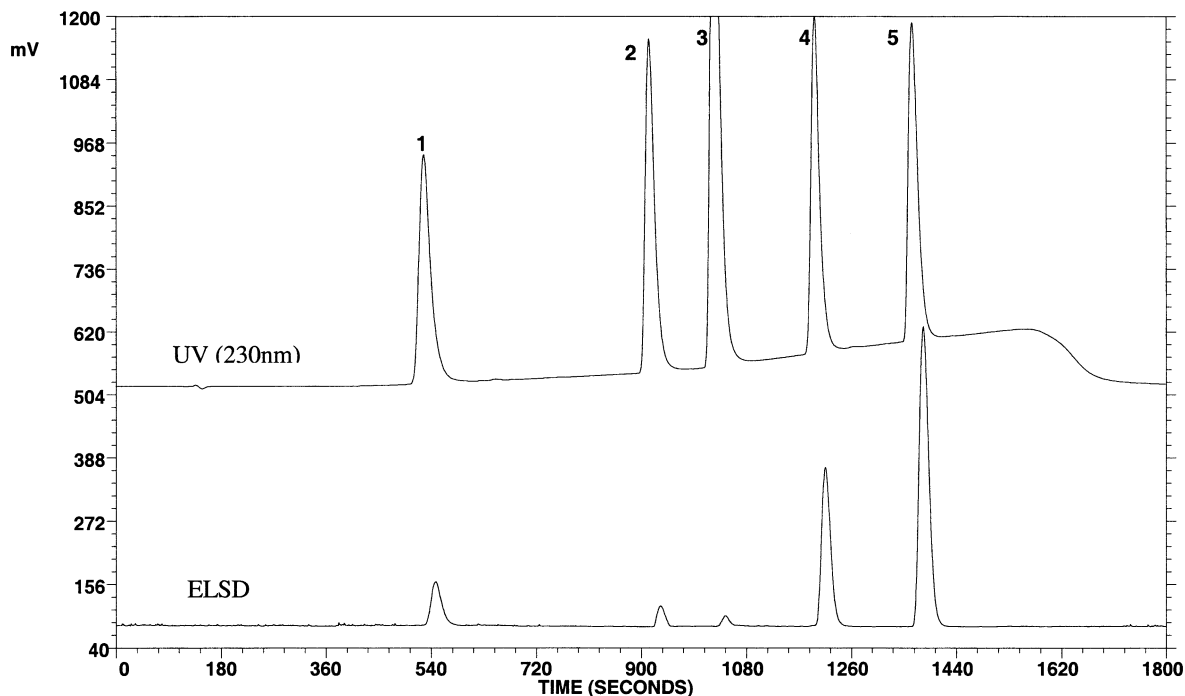[b] HPLC conditions (RRF$_2$): acetonitrile/water/pH 3.0 phosphate.

Fig. 4. UV (230nm) and ELSD detection of test mixture (1, acetaminophen, 0.31 mg/ml; 2, 2-quinoxalinol, 0.15 mg/ml; 3, 2-hydroxyquinoline, 0.29 mg/ml; 4, dansyl-phenylalanine, 0.40 mg/ml; 5, fluoxetine, 0.61 mg/ml); HPLC conditions as in Fig. 3. ELSD drift tube, 65 °C; gas flow, 3 l/min.

to detect 2-hydroxyquinoline at all. Even so, some of the compounds gave a much smaller ELSD response per unit weight than others. Table 2 lists the apparent RRF values obtained using the ELSD peak areas in place of sample weights (i.e. assuming ELSD response is proportional to sample weight). Clearly, the ELSD response is not uniformly related to relative weight under these conditions and cannot be used to obtain accurate RRF data for these dissimilar compounds.

3.2. Mass balance of photodegraded nifedipine

Nifedipine (4-(2′-nitrophenyl)-2,6-dimethyl-3,5-dimethoxycarbonyl-1,4-dihydropyridine) is a well-characterized light-sensitive pharmaceutical compound. Upon exposure to sunlight or even room light, it rapidly oxidizes in solution to form 4-(2′-nitrosophenyl)-pyridine (see structures in Fig. 5) [14]. This degradation product has a significantly different UV absorption spectrum than nifedipine. As a result, the relative response factors of the two compounds are dissimilar at most wavelengths. Thus, quantitation of nifedipine and its degradation product by HPLC-UV is susceptible to mass-balance errors unless these RRF values are taken into consideration. Use of the CLND allows one to determine both the true mass balance and the RRF values, as demonstrated below.

A sample of nifedipine (0.17 mg/ml in 60/40 MeOH/H_2O) with minimal exposure to light was assayed ($n = 6$) at its λ_{max} (237 nm) in order to get a precise measure of the CLND and UV peak areas for the parent compound. The sample was then degraded by exposing to intense light from a projector lamp over the course of 6 h. Aliquots were assayed regularly throughout this time period by HPLC-UV–CLND. After 6 h of degradation, the lamp was turned off and the sample was assayed in duplicate at this final time-point. Sample chromatograms showing the progress of conversion to the oxidation product are shown in Fig. 6. Each peak was integrated with both the UV and CLND detectors.

With UV detection at 237 nm, the total peak area (i.e. sum of the peak areas of nifedipine and its oxidation product) decreased throughout the photodegradation of nifedipine. For example, the total UV peak area after 6 h was only 64% of its initial value. However, the total peak area by CLND was consistent throughout, with an overall R.S.D. of 2.5%. Thus the CLND clarifies that the decrease in total UV peak area is due to different RRF values for the two compounds. Furthermore, the RRF value for the degradation product is readily calculated using Eq. (1).

Table 2
RRF at 230 nm as determined from known compound weights or from ELSD peak areas

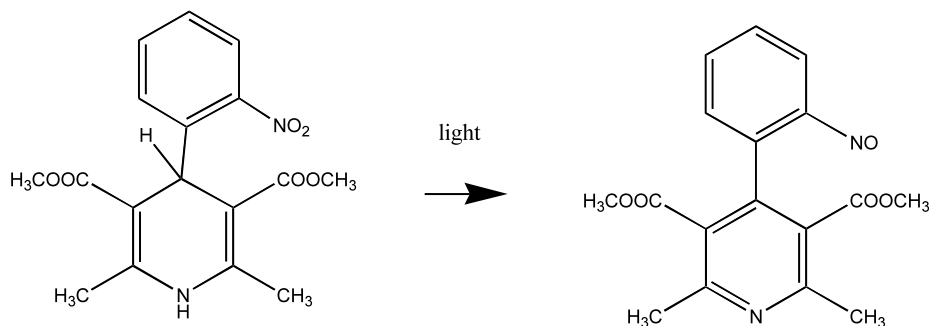| Compound	RRF by known weights[a]	RRF by ELSD[a]	% ELSD accuracy
Acetaminopen	2.63	5.61	213
2-Quinoxalinol	4.64	18.3	394
2-Hydroxyquinoline	4.15	72.1	1740
Dansyl-Phenylalanine	1.46	1.95	134
Fluoxetine	1.00 (reference)	1.00 (reference)	N/A

[a] HPLC conditions: methanol/water/0.1% TFA.

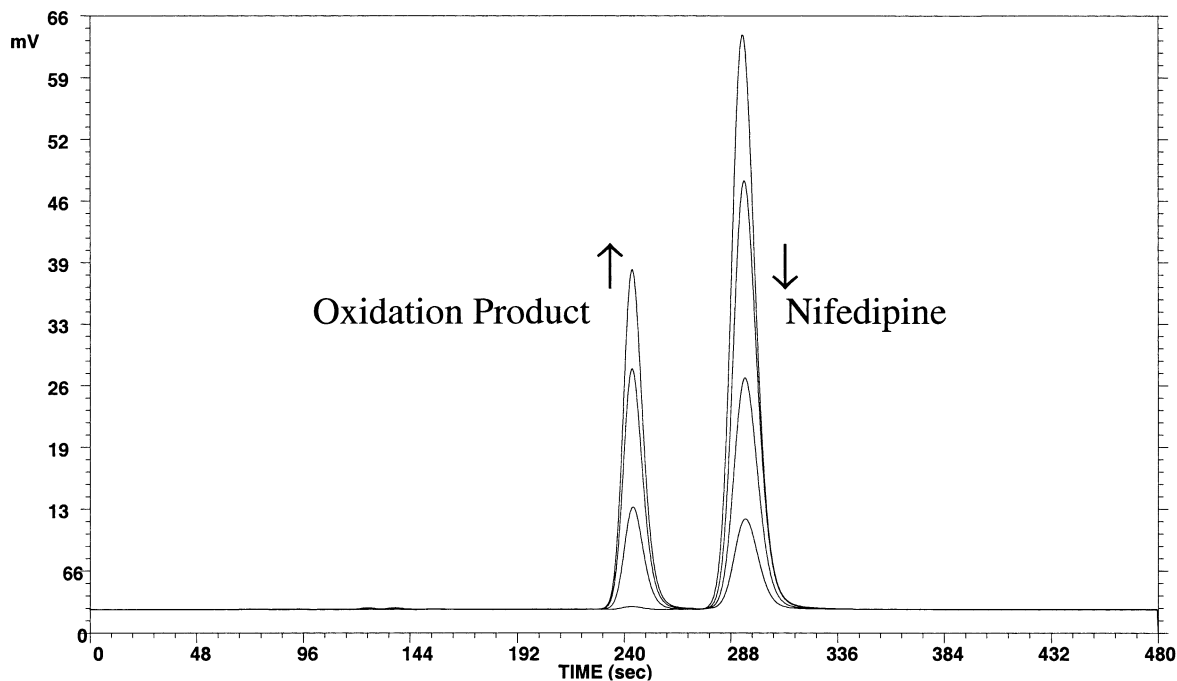Fig. 5. Photodegradation of nifedipine [14].

Fig. 6. Photodegradation of nifedipine as monitored by HPLC/UV. HPLC conditions: Zorbax Rx-C18, 0.21 × 15 cm; mobile phase: 60/40 MeOH/H$_2$O, 0.2 ml/min; 5 µl injection. Detection: UV, 237 nm.

$$RRF_{237 \ nm} = \frac{(12981/7405) \times (328.3)/2}{(26847/8620) \times (346.3)/2} = 0.534$$

on a weight basis.

Omitting the molecular weight information gives the RRF value on a molar basis: 0.563. By using this RRF value, the UV results can be readily corrected.

Finally, the appropriate RRF for non-CLND-compatible conditions can be determined and used with other HPLC instruments. For example, the USP monograph assay for nifedipine uses a mobile phase of water:acetonitrile:methanol (50:25:25). By running a partially degraded sample via HPLC-UV under both sets of conditions, the molar RRF$_2$ value for the USP monograph conditions was calculated from Eq. (2) to be 0.627. Thus, peak areas obtained for the degradation product using these conditions can be corrected by dividing by 0.627. As a test case, initial, partially and fully degraded nifedipine samples were run under the USP HPLC conditions. The corrected and uncorrected results are shown in Table 3. The mass balance is simply the total peak

area at a given time, divided by the initial total area.

Note that, without RRF correction, the apparent mass balance is quite poor, dropping to 65% for a fully degraded sample. Thus, in the absence of RRF information, one might conclude that mass balance was not achieved and that other, undetected degradation products were being formed or that parent compound was being lost (e.g. via adsorption, volatilization). Use of the RRF$_2$ value above, however, provides excellent mass balance, even for a fully degraded sample.

4. Conclusions

The CLND provides a new and simple alternative to the need for purified samples for determination of relative UV response factors. We have found the CLND results to be accurate to within 10%, even for compounds with widely varying structures and across gradient HPLC conditions. This level of accuracy, while perhaps insufficient

Table 3
Mass balance of photodegraded nifedipine: before and after RRF correction from CLND data

Sample	Nifedipine peak area	Ox. product raw peak area	Total peak area	Apparent mass balance (%)	Ox. product corrected area	Total peak area	Corrected mass balance (%)
Initial	13 658	105	13 763	n/a	168	13 826	n/a
Partially degraded	7558	4070	11 628	84.5	6491	14 049	101.6
Fully degraded	16	8977	8993	65.3	14 318	14 334	103.7

for some needs, is more than sufficient for the needs of RRF determinations of impurities in drug substances and products. Thus the CLND should prove very helpful for establishing appropriate detection wavelengths and/or correction factors for accurate assessments of purity of pharmaceuticals and other compounds of interest. The CLND can also help resolve issues related to mass balance; for example, when degradation products appear to exceed or fall short of 100% mass balance due to RRF differences. Conversely, the CLND may indicate a true mass balance issue (i.e. some degradation product(s) unaccounted for) in instances where higher-than-expected RRF values of the integrated degradation products would otherwise mask the fact that additional degradation products exist.

Further improvements in the precision and reliability of the CLND as this technology develops should facilitate expanded applications. The model of instrument used in this work is complex and relatively new, and as such, is not as rugged as UV or other standard detectors. Flow rates are limited to less than 400 μl/min, and the nebulizer can become clogged or inorganic deposits can build up downstream of the furnace. Also, peak tailing can be much more significant than with UV; this, along with baseline noise, makes integration of peaks less precise. Finally, samples of interest must contain nitrogen in large enough amounts to be accurately integrated (typically ≥ 1 ng nitrogen injected). However, the CLND presents a new and powerful tool for quantitation, which is unavailable in other HPLC detectors, and as such, offers substantial potential. Determination of relative UV response factors and addressing mass balance concerns, as demonstrated here, are but two broadly-applicable examples.

References

[1] M. Righezza, G. Guiochon, J. Liq. Chromatogr. 11 (1988) 1967–2004.
[2] V.L. Cebolla, L. Membrado, J. Vela, A.C. Ferrando, Semin. Food Anal. 2 (1997) 171–189.
[3] A.I. Hopia, V.M. Ollilainen, J. Liq. Chromatogr. 16 (1993) 2469–2482.
[4] T.J. McNabb, A.E. Cremesti, P.R. Brown, A.A. Fischl, Semin. Food Anal. 4 (1999) 53–70.
[5] C.E. Kibbey, Mol. Diversity 1 (1995) 247–258.
[6] B.H. Hsu, E. Orton, S. Tang, R.A. Carlton, J. Chromatogr. B 725 (1999) 103–112.
[7] L. Fang, M. Wan, M. Pennacchio, J. Pan, J. Comb. Chem. 2 (2000) 254–257.
[8] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC Method Development, second ed., Wiley, New York, 1997, pp. 80–81.
[9] W.L. Fitch, A.K. Szardenings, E.M. Fujinari, Tetrahedron Lett. 38 (1997) 1689–1692.
[10] E.W. Taylor, M.G. Qian, G.D. Dollinger, Anal. Chem. 70 (1998) 3339–3347.
[11] N.J. Haskins, C. Eckers, A.J. Organ, M.F. Dunk, B.E. Winger, Rapid Commun. Mass Spectrom. 9 (1995) 1027–1030.
[12] G. Perkins, F. Pullen, C. Thompson, J. Am. Soc. Mass Spectrom. 10 (1999) 546–551.
[13] M.E. Palmer, M.R. Clench, L.W. Tetler, D.R. Little, Rapid Commun. Mass Spectrom. 13 (1999) 256–263.
[14] P. Pietta, A. Rava, P. Biondi, J. Chromatogr. 210 (1981) 516–521.