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ScienceDirect
Journal of Chromatography A, 1177 (2008) 87–91
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of relative response factors of impurities in paclitaxel with high performance liquid chromatography equipped with ultraviolet and charged aerosol detectors

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Received 28 August 2007; received in revised form 25 October 2007; accepted 5 November 2007
Available online 19 November 2007

Abstract

A case study was conducted to determine the relative response factors (RRFs) of paclitaxel-related impurities by high performance liquid chromatography (HPLC) equipped with an ultraviolet (UV) detector and charged aerosol detector (CAD) in tandem. The peak response using CAD was independent of analyte structure in an isocratic analysis for this application. After a sample containing known and unknown impurities was analyzed with HPLC–UV–CAD, an empirical approach was developed to calculate the RRFs for all impurities. The RRFs of known impurities were also determined by linear calibration curves. For known impurities, the RRFs values determined with two approaches are comparable. The new approach is effective yet simpler to determine the RRFs for unknown impurities or degradation products since the need for obtaining authentic pure materials was eliminated.

Keywords: HPLC; Charged aerosol detector (CAD); Ultra-violet (UV); Relative response factors (RRFs); Paclitaxel

1. Introduction

HPLC–UV is one of the most successful tools in pharmaceutical analysis, due to its sensitivity, reproducibility, and simplicity [1–5]. One popular application is the purity determination of drugs, which is a crucial issue in pharmaceutical discovery and development. Evaluation of compound purity using HPLC–UV involves LC separation and quantitation of detected peaks at a specific wavelength.

In this process, the determination of a UV relative response factors (RRFs) at a specific wavelength is required for quantitative analysis. The conventional way to determine UV RRFs is to analyze two pure compounds (typically one is the impurity and the other is the active pharmaceutical ingredient) in defined quantities under the same detection conditions and calculate ratio of slopes of the linear calibration curves. This approach, however, is not achievable for unknown impurities when authentic materials of known purity are not readily available. An acceptable alternative to determine the UV RRFs is to use two detectors in tandem: a UV detector and a universal detector. The ideal universal detector should provide a consistent relationship between response magnitude and quantity of the injected compound, which is independent of compound identity.

Some HPLC detectors, such as refractive index (RI), evaporative light scattering detector (ELSD), and mass spectrometry (MS) are candidates for universal detectors. Each of these techniques has weaknesses, limiting its application as a real universal HPLC detector. RI has problems in sensitivity and reproducibility and it cannot be applied in gradient analysis which is standard practice in pharmaceutical analysis [6–8]. Although ELSD response is less affected by compound structure than UV, issues regarding precision, and dynamic range, and truly consistent response [9–13] limit its utility. MS is widely used for its good sensitivity and proficient structure information. However, its response is variable to different analytes due to their differences in ionization efficiency. Recently, a chemiluminescent nitrogen-specific detector (CLND) was reported to determine UV relative response factors [14]. The CLND response is pro-

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doi:10.1016/j.chroma.2007.11.035

portional to nitrogen amount, giving accurate concentration if the molecular formulas are known. The limitation of this detector is that it only responds to molecules containing nitrogen. Also, this detection device is not compatible with nitrogen-containing solvents, such as triethylamine and acetonitrile, which are common reversed phase HPLC solvents.

Recently, a new universal HPLC detector has been developed based on charged aerosol detection (CAD) [15–18]. CAD has proven to be a powerful new detection technology because of unique performance characteristics: better sensitivity than ELSD, a dynamic range of up to 4 orders of magnitude, ease of use, and constancy of response factors. Since CAD shares some elements of ELSD, it also has the same limitation as ELSD, namely, the response is affected by mobile phase composition. Fortunately, this problem in CAD application has been resolved by the use of inverse gradient compensation as is done for HPLC and SFC detectors [19,20].

Paclitaxel, first extracted from the bark of the Pacific yew tree, has developed into one of the most widely used anticancer drugs today and thousands of papers have been published on various areas related to paclitaxel [21–26]. It has received considerable clinical attention because it has been demonstrated to be effective against a broad range of tumor types, including breast, ovarian, head and neck, and non-small-cell lung cancers [27–30]. Paclitaxel is a diterpene member of the taxane family of natural products and many structure-related taxane compounds are present in raw materials, which leads to interest in separation and quantitation of these related compounds. The objective of this study was to develop HPLC–UV–CAD method to determine UV RRFs of impurities in paclitaxel.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile and water of HPLC grade were purchased from EMD (Gibbstown, NJ) and J.T. Baker (Phillipsburg, NJ), respectively. 50/50 poly(DL-lactide-*co*-glycolide) (PLGA) was purchased from Durect Corp. (Pelham, AL). Dimethyl sulfoxide (DMSO) was obtained from Burdick & Jackson (Muskegon, MI). Paclitaxel and eight related compounds of reagent grade were obtained from InB: Hauser Pharmaceutical Services (Denver, CO). These compounds are 10-deacetylbaccatin III (compound **1**), baccatin III (compound **2**), 10-deacetyl-7-xylosyltaxol C (compound **3**), photodegradant (compound **4**), cephalomannine (compound **5**), 10-deacetyl-7-epitaxol (compound **6**), paclitaxel (compound **7**), taxol C (compound **8**), and 7-epitaxol (compound **9**).

2.2. Equipment

The assay utilized a HPLC 1200 Series system from Agilent Technologies (Santa Clara, CA). In all experiments, the Corona CAD (ESA Biosciences Inc., Chelmsford, MA) was connected in series after the diode array detector of 1200 Series. UV–vis and CAD signals were obtained simultaneously. Curosil-PFP (pentafluorophenyl) columns (150 mm × 4.6 mm, 3 μm) were purchased from Phenomenex (Torrance, CA). The nitrogen generation system (Model N2-14) is from Parker Hannifin Corp. (Haverhill, MA).

The column temperature was kept at 35 °C. The injection volume, mobile phase, and flow rate were 50 μL, 52/48 (water/acetonitrile), and 1.2 mL/min, respectively, unless otherwise noted. The UV absorbance was monitored at 227 nm and the bandwidth was 4 nm. The CAD nitrogen gas pressure was adjusted to 35 psi. A 100 pA output range and high filter were used for CAD monitoring.

2.3. Sample preparation

Sample solvent was prepared by mixing 600 mL water and 400 mL acetonitrile. Paclitaxel (compound **7**) solutions were prepared at nominal concentrations of 0.1 μg/mL, 0.5 μg/mL, 1 μg/mL, 2 μg/mL, 25 μg/mL, 50 μg/mL, 75 μg/mL, 100 μg/mL, 125 μg/mL, and 150 μg/mL in sample solvent. Likewise, solutions of the other compounds (compounds **1–6**, **8–9**) were prepared at four nominal concentrations (0.1 μg/mL, 0.5 μg/mL, 1.0 μg/mL, and 2.0 μg/mL) in sample solvent and a mixture containing nine compounds at approximately 10 μg/mL was prepared in sample solvent. A stressed sample was prepared by adding 50 μL DMSO to approximately 45 mg paclitaxel and 400 mg PLGA dissolved in acetonitrile, evaporating off acetonitrile and heating the mixture at 60 °C for 48 h. Then, the stressed sample was dissolved in 25 mL acetonitrile and ready for LC injection. The injection volume for the stressed sample solution is 5 μL and the mobile phase is 60/40, water/acetonitrile.

3. Results and discussion

3.1. Determination of RRFs with linear calibration curves

Linear calibration curves for all nine compounds were constructed using the peak areas and analyte concentrations in the range of 0.1–2.0 μg/mL by linear regression analysis. The linearity data of all analytes were summarized in Table 1. The linearity of paclitaxel in high concentration ranges (25–150 μg/mL) was also studied. The regression equation was calculated as $y = 84.097x + 70.663$. The slope of the

Table 1
Regression data and UV relative response factors of paclitaxel-related compounds

Compound	Regression equation[a]	r^2	RRF_{uv}[b]
10-Deacetylbaccatin III	$y = 66.160x + 0.8502$	0.9992	0.79
Baccatin III	$y = 57.899x + 0.3943$	0.9999	0.69
10-Deacetyl-7-xylosyltaxol C	$y = 39.205x - 0.0477$	1.0000	0.47
Photodegradant	$y = 61.839x + 0.3769$	0.9999	0.74
Cephalomannine	$y = 67.346x + 0.5839$	0.9999	0.80
10-Deacetyl-7-epitaxol	$y = 75.447x + 0.9273$	0.9997	0.90
Paclitaxel	$y = 84.150x - 1.0984$	0.9988	1.00
Taxol C	$y = 42.073x + 0.1346$	0.9999	0.50
7-Epi-taxol	$y = 77.381x + 0.7795$	0.9998	0.92

[a] The regression curves were obtained by plotting concentration (x) vs. peak area (y). R^2 is the coefficient of determination.
[b] RRF_{uv} is UV relative response factor to paclitaxel at 227 nm.