Journal of Pharmaceutical and Biomedical Analysis 51 (2010) 973–978

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Comparison of ultraviolet detection, evaporative light scattering detection and charged aerosol detection methods for liquid-chromatographic determination of anti-diabetic drugs

Jia Shaodong[a], Won Jun Lee[a], Ji Won Ee[a], Jeong Hill Park[a], Sung Won Kwon[a,*], Jeongmi Lee[b,*]

[a] College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 151-742, Republic of Korea
[b] College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

ARTICLE INFO

Article history:
Received 15 June 2009
Received in revised form 19 October 2009
Accepted 21 October 2009
Available online 31 October 2009

Keywords:
Glipizide
Gliclazide
Glibenclamide
Glimepiride
HPLC
ELSD
CAD
Pharmaceutical analysis

ABSTRACT

Recently, charged aerosol detection (CAD), a new kind of universal detection method, has been widely employed in the HPLC system. In the present study, four kinds of anti-diabetic drug standards, glipizide, gliclazide, glibenclamide and glimepiride were determined by ultraviolet (UV) detection, evaporative light scattering detection (ELSD) and the aforementioned CAD. The results were compared with reference to linearity, accuracy, precision and limit of detection (LOD). All of the experiments were performed on a reverse phase column with water and acetonitrile as the mobile phase. Separations were achieved under the same chromatographic conditions for each detection method. As a result, CAD generated nearly uniform responses compared with UV detection and ELSD. It showed the best accuracy and LOD among 3 detectors and had similar precision with UV detection at higher concentrations while UV detection showed a better precision at lower concentrations than did CAD or ELSD. The LOD of CAD, in fact, can be up to two times higher than that of ELSD. The UV and ELSD linearity was satisfactory at $R^2 > 0.99$, though in the case of CAD, a log–log transformation was needed. The proposed methods were also applied to the real anti-diabetic drugs and diabetes-related dietary supplements.

1. Introduction

Ultraviolet (UV) detection is the most popular detection method for liquid chromatography in the pharmaceutical industry, due to its high sensitivity, broad linear range, ease of operation and other advantages, as well as its compatibility with most mobile phase solvents. However, it requires a tedious and time-consuming derivatization procedure unless pharmaceutical compounds possess a UV-absorbing chromophore. Techniques such as refractive index (RI) detection or mass spectrometry (MS) detection have been employed for the detection of UV-undetectable compounds. But RI detection has the disadvantages of low sensitivity and incompatibility with gradient elution; MS detection, moreover, is expensive for routine use, and its requirement of specially trained operators limits its applicability further. Evaporative light scattering detection (ELSD) and, charged aerosol detection (CAD), introduced more recently, are additional alternatives to UV detection. Consequently, the response generated by CAD and ELSD are independent of the chemical structures of the compounds [1]. ELSD and CAD, theoretically, offer similar responses for the same mass of analytes since their response is mass-dependent in contrast to that of UV detection, which is concentration-dependent.

ELSD [2–4] has gained great popularity for the detection of compounds that are nonvolatile or lacking in UV-absorbing chromophores; however, in some cases, unsatisfactory quantitativeness, reproducibility, sensitivity and dynamic range have been reported [5,6], and its response varied with the solvent composition [7,8]. CAD, introduced by Dixon and Peterson [9], is considered to be more sensitive than ELSD [10]. The CAD process can be described briefly as follows: column eluents are nebulized by a stream of nitrogen; they are evaporated through a drift tube to produce dried analyte particles; the dried particles are charged by a secondary stream of nitrogen, which is positively charged by a high voltage wire; finally, the eluent's electric charge is transferred to a collector and measured via an electrical aerosol analyzer, the signal being in direct proportion to the mass of the analyte particles [9,11]. CAD shares the limitations of ELSD, in that the response varies with the composition of the mobile phase, and that peak areas can be increased with the increase of organic additives in the mobile phase when gradient elution is applied. Fortunately, this drawback was overcome by means of an inverse gradient compensation technique [11,12]. CAD, due to the sensitivity, reproducibility and accuracy of its analytical quantification, has been widely employed in analytical

* Corresponding authors. Tel.: +82 31 290 7703/+82 2 880 7844; fax: +82 2 886 7844.
E-mail addresses: swkwon@snu.ac.kr (S.W. Kwon), jlee0610@skku.edu (J. Lee).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.10.019

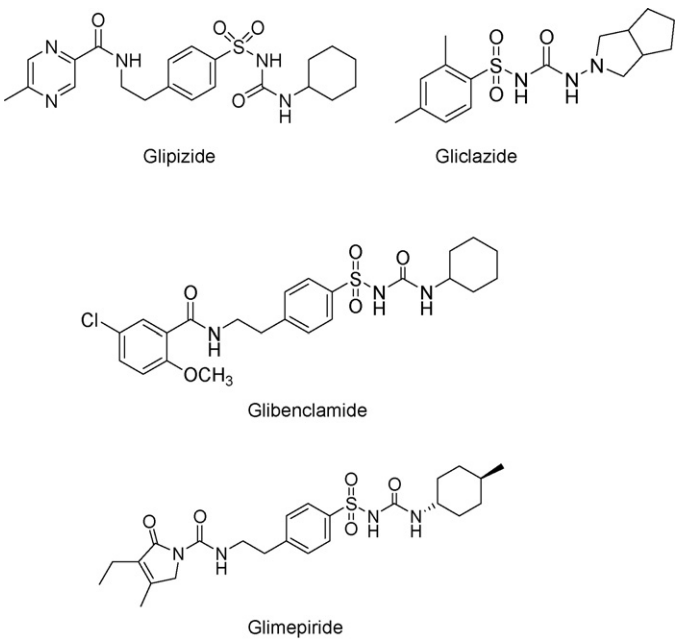

Fig. 1. Structures of four anti-diabetic drugs.

tasks such as synthetic polymer determination [13], lipid compound analysis [14–17], evaluation of triacylglycerols from plant oils [18], enantiomeric ratio determination [19] and simultaneous analysis of ascorbic acid and dehydroascorbic acid [20]; lately, moreover, it has become a more attractive detection method in the pharmaceutical application field [1,21].

Anti-diabetic drugs such as metformin, glipizide, gliclazide, glibenclamide and glimepiride are commonly used in the treatment of type II diabetes [22]. The structures of the last four of those drugs are shown in Fig. 1. Methods for the determination of single drug in serum or urine are well established [23–26]. These days multiple drug therapy is sometimes applied in order to keep the disease under control [22] and therefore several methods for simultaneous detection of multiple drugs have been developed. Paroni et al. applied capillary electrophoresis (CE) to determine chlorpropamide, tolbutamide, glipizide, gliclazide, and glibenclamide in serum [27]. Vasudevan et al. developed an ion-pair HPLC method for the determination of metformin with gliclazide and glipizide in multicomponent dosages [28]. Sener et al. determined glibenclamide, gliquidone, glipizide, or gliclazide in plasma using HPLC and other methods [29]. However, all of these methods were developed for use with only UV detection. In the present study, we compared the UV, ELSD and CAD methods for the detection of glipizide, gliclazide, glibenclamide and glimepiride, with reference to linearity, accuracy, precision and limit of detection. And the three detection methods were applied to the analysis of real commercial drugs and anti-diabetic dietary supplements in an attempt to screen the counterfeit drug or the illegal adulterants which may exist in dietary supplements. There have been few papers on the pharmaceutical applications of CAD, and additionally, to the best of our knowledge, ours is the first comparison among the three detection methods for anti-diabetic drugs and dietary supplements. This work could provide other researchers with a new alternative method of anti-diabetic drug-related research.

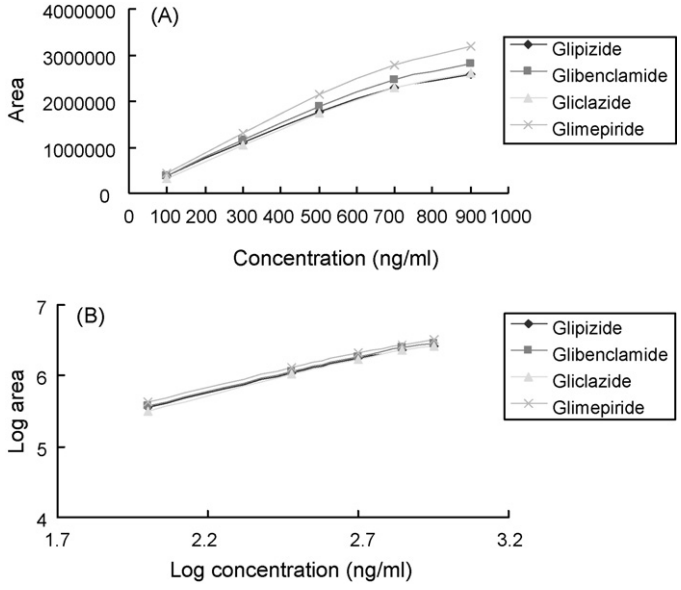

Fig. 2. Linear curves of CAD: (A) linear coordinates; (B) logarithmic coordinates.

2. Materials and methods

2.1. Chemicals and reagents

Purified water, acetonitrile and methanol of HPLC grade were purchased from Duksan Pure Chemicals (Ansan, Korea). Glipizide, gliclazide, glibenclamide, and glimepiride standards were all purchased from Sigma–Aldrich (St. Louis, USA). The dietary supplements A (product A) was purchased from a market in Canada and B (product B) and C (product C) from China. The commercial drug tablets were all purchased from Korea. The standard stock solutions were prepared by dissolving glipizide, gliclazide, glibenclamide, and glimepiride in methanol to obtain the desired concentration. For the calibration curves, stock solutions were further diluted by methanol to obtain five concentrations (10, 30, 50, 70 and 90 μg/mL). Drugs and dietary supplements in tablet or capsule were powered, weighed and triturated to get homogeneous mixtures. Drug solutions were made by dissolving a certain amount of each powder in methanol to contain 50 μg/mL of active component. Dietary supplement powder was extracted in methanol by sonication for 10 min and made to a final concentration of 2 mg/mL.

2.2. Instrumentation

The Series 200 HPLC system (PerkinElmer, USA) was used in all of the experiments. The system consists of a PerkinElmer Series 200 pump and an auto-sampler. Totalchrom Workstation software was used for the data collection and processing. Detection was accomplished using UV (PerkinElmer Series UV/Vis), ELSD 2000 (Alltech Associates, Deerfield, IL, USA), and corona CAD plus (ESA, Chelmsford, MA, USA) detectors.

2.3. Chromatographic conditions

A GraceSmart RP-18 packed column (250 mm × 4.6 mm, 5 μm) was used for the HPLC separation. An isocratic elution system consisting of 35% A (90% water + 0.1% formic acid + 10% acetonitrile) and 65% B (90% acetonitrile + 10% water) was developed. The injection volume was 10 μL and each injection was repeated three times. The flow rate of the mobile phase was maintained at 1 mL/min. The experiments were carried out at room temperature.