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Short communication

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

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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

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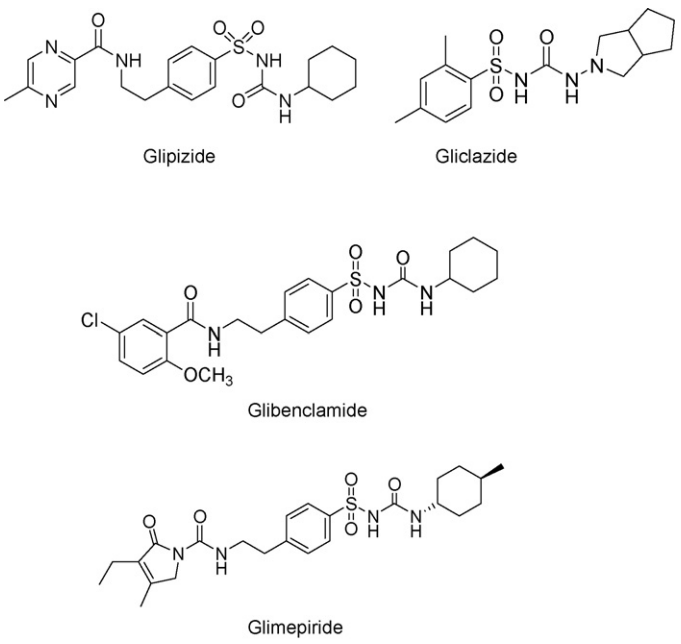

Fig. 1. Structures of four anti-diabetic drugs.

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.

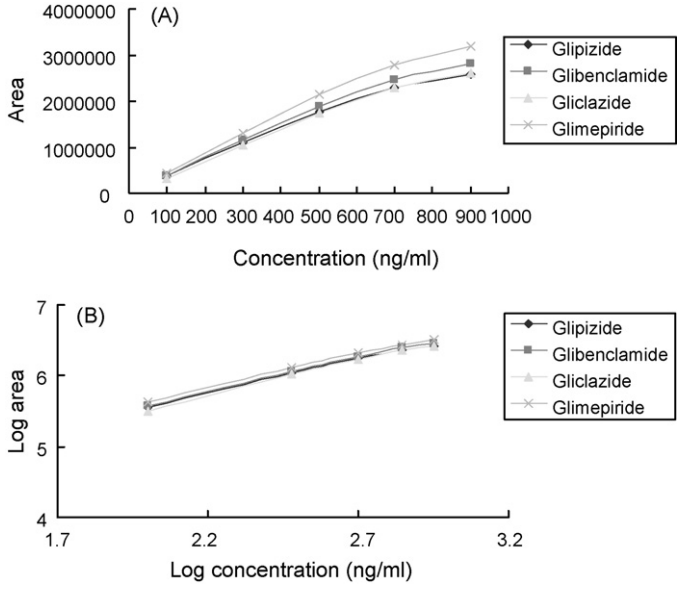

Fig. 2. Linear curves of CAD: (A) linear coordinates; (B) logarithmic coordinates.

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.

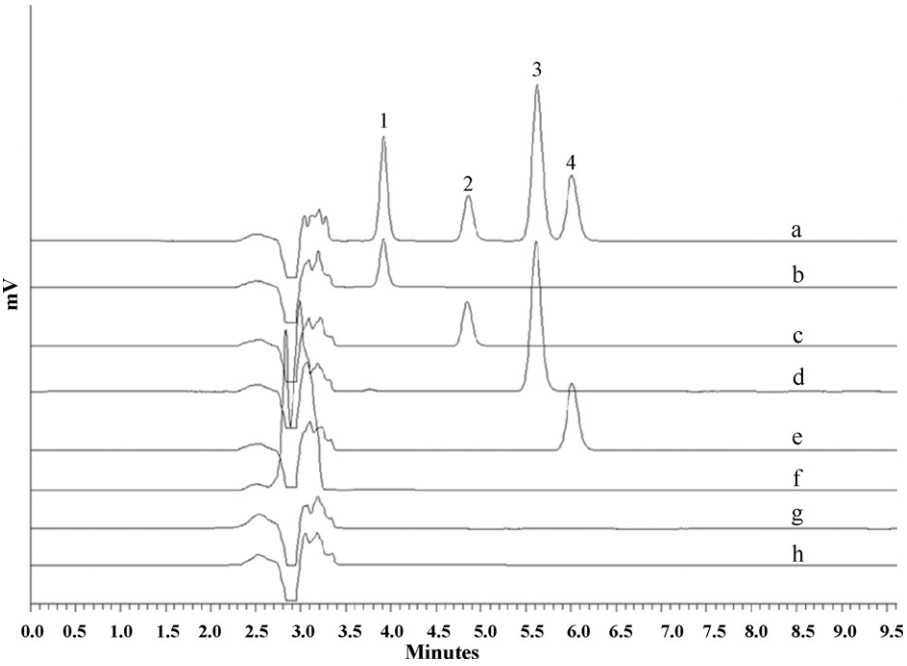

Fig. 3. Chromatogram of HPLC/UV detection (a) standard mixture; 1, glipizide; 2, gliclazide; 3, glibenclamide; 4, glimepiride; (b) glipizide tablet; (c) gliclazide tablet; (d) glibenclamide tablet; (e) glimepiride tablet; (f) product A; (g) product B; (h) product C.

2.4. CAD and ELSD parameters

The range of the corona CAD detector was set to 100 pA, and the inlet nitrogen pressure was set at 35 psi, according to the operating instruction. The nebulizer gas for ELSD was nitrogen, and the gas flow rate was set at 2.3 L/min. The ELSD gain was 8, the drift tube temperature was 88 °C, and the "impactor off" mode was applied. The wavelength of the UV detector was adjusted to 210 nm for all of the drug detections.

2.5. Method validation

Linearity, precision, accuracy and limit of detection (LOD) were used as the validation parameters for the three detection methods. The linearity was investigated for the calibration curves in which the 5 concentrations (10, 30, 50, 70 and 90 μg/mL) were plotted the corresponding peak areas. The accuracy was tested by injecting 10, 50, and 90 μg/mL of each standard into the detectors and comparing the peak areas with those from the calibration curves.

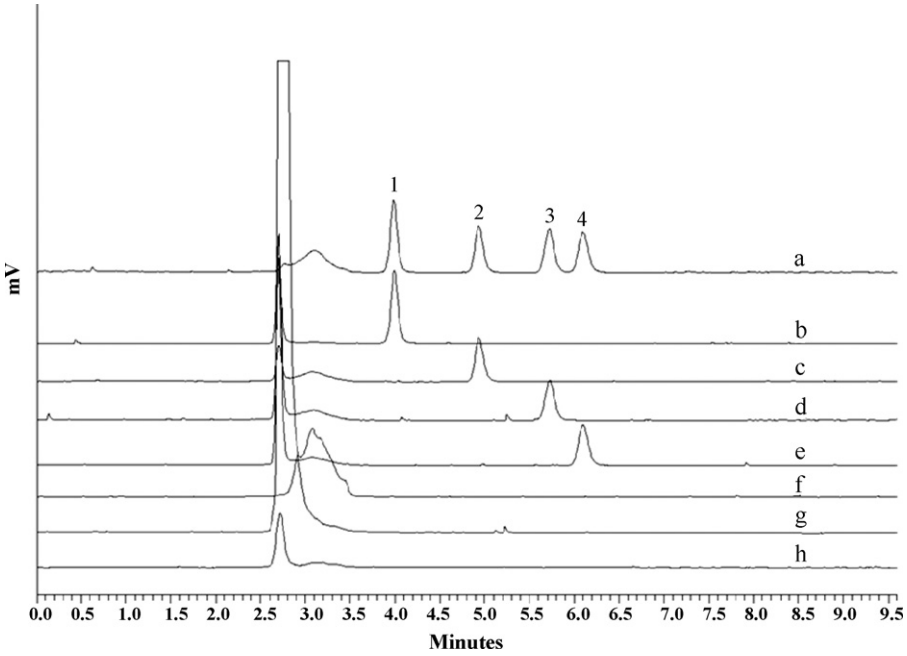

Fig. 4. Chromatogram of HPLC/ELSD detection (a) standard mixture; 1, glipizide; 2, gliclazide; 3, glibenclamide; 4, glimepiride; (b) glipizide tablet; (c) gliclazide tablet; (d) glibenclamide tablet; (e) glimepiride tablet; (f) product A; (g) product B; (h) product C.

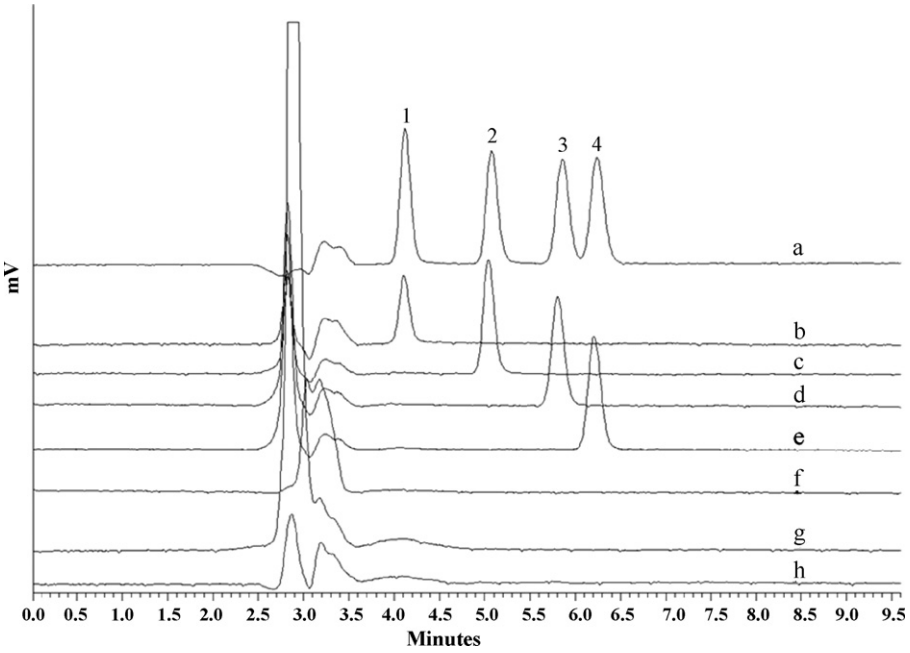

Fig. 5. Chromatogram of HPLC/CAD detection (a) standard mixture; 1, glipizide; 2, gliclazide; 3, glibenclamide; 4, glimepiride; (b) glipizide tablet; (c) gliclazide tablet; (d) glibenclamide tablet; (e) glimepiride tablet; (f) product A; (g) product B; (h) Product C.

For the recovery, 500 μg/mL of each standard was spiked to the methanol extract of a dietary supplement (product A, Canada) and the recovery was calculated according to the following equation; recovery (%) = experimental amount/spiked sample amount × 100. The precision was calculated by relative standard deviation (RSD). Standard solutions of five different concentrations were tested three times for intra-day and inter-day precision.

Limit of detection (LOD) was acquired using the formula; LOD = 3.3σ/S, where σ is standard deviation of the response and S is the slope of calibration curve.

Table 1
Accuracy and recovery for UV detection, CAD and ELSD.

	Accuracy (%)		
	100 ng[a]	500 ng	900 ng
UV detection			
Glipizide	98.0	102.4	99.3
Gliclazide	100.04	102.5	98.7
Glibenclamide	98.9	102.5	98.5
Glimepiride	98.6	101.6	98.9
CAD			
Glipizide	102.5	104.3	101.8
Gliclazide	106.1	104.7	101.3
Glibenclamide	99.4	102.5	98.1
Glimepiride	99.6	101.6	99.0
ELSD			
Glipizide	123.3	103.2	97.2
Gliclazide	106.5	107.4	100.5
Glibenclamide	166.0	105.9	100.2
Glimepiride	104.8	107.6	99.3
	Recovery (%)		
	UV	CAD	ELSD
Glipizide	93.2	97.0	83.1
Gliclazide	87.3	96.1	78.5
Glibenclamide	91.8	98.0	81.7
Glimepiride	91.0	98.4	83.5

[a] The 'ng' was calculated by each concentration × injection volume (10 μL).

3. Results and discussion

3.1. Optimization of mobile phase

Organic acids were usually added into the mobile phase to enhance separation and sensitivity. In the attempt to optimize mobile phase consisting of water and acetonitrile, 0.1% trifluoroacetic acid, 0.1% acetic acid and 0.1% formic acid were tested in order to optimize the mobile phase consisting of water and acetonitrile for the analysis of 4 anti-diabetic drug compounds. 0.1% formic acid yielded the best peak shapes and peak heights among the tested additives (data not shown). Accordingly, 0.1% formic acid was chosen as the mobile phase additive for all the experiments. In this study, the analysis of glipizide, gliclazide, glibenclamide and glimepiride could be conducted in less than 8 min by any of the three selected methods of detection. The typical chromatograms were shown in Fig. 3, 4 and 5. By contrast with UV detection and ELSD, CAD generated nearly uniform response of peak areas for the four drugs. Based on this feature, CAD is suitable for simple and fast semi-quantitation of those drugs without the preparation of standard calibration solutions.

3.2. Linearity

Some studies have shown that ELSD is non-linear, but in the present work, both UV detection and ELSD ($R^2 > 0.99$) were quite acceptable for quantitative purposes. In the case of CAD, a log–log transformation for the calibration curve was necessary, as the linearity ($R^2 < 0.99$) was not quite satisfactory, which judgment concurred with those of other reports [1,11,12]. As can be seen in Fig. 2, after the log–log plot of the peak area versus sample concentration, a linear curve ($R^2 > 0.99$) could be achieved.

3.3. Accuracy and precision

The accuracies of UV, CAD and ELSD detection were all acceptable for the concentration of 50 and 90 μg/mL with the value around 100%. ELSD showed worse result for the test of 10 μg/mL with the

Table 2
Relative standard deviation (%) for UV detection, CAD and ELSD with 5 drug concentrations ($n = 3$).

	Intra-day test				
	100 ng	300 ng	500 ng	700 ng	900 ng
UV detection					
Glipizide	0.3	0.1	0.4	0.2	1.6
Gliclazide	0.6	0.4	0.2	0.3	0.2
Glibenclamide	0.2	0.1	0.4	0.5	0.3
Glimepiride	0.3	0.2	0.7	0.9	0.6
CAD					
Glipizide	6.2	1.9	2.6	2.3	2.8
Gliclazide	8.7	2.0	2.5	2.9	3.7
Glibenclamide	9.5	2.0	2.3	2.5	3.8
Glimepiride	8.7	2.1	2.0	2.6	3.9
ELSD					
Glipizide	14.0	8.3	4.0	5.7	4.9
Gliclazide	4.3	4.0	5.6	4.6	5.9
Glibenclamide	26.6	5.1	2.6	4.8	6.4
Glimepiride	10.5	5.0	6.4	6.9	5.1
	Inter-day test				
UV detection					
Glipizide	0.9	1.6	2.5	0.5	0.8
Gliclazide	0.6	1.0	2.2	2.2	2.2
Glibenclamide	0.3	1.1	2.0	2.0	1.6
Glimepiride	0.6	1.3	1.9	1.8	1.1
CAD					
Glipizide	4.9	2.6	3.6	1.5	1.2
Gliclazide	8.2	6.4	3.4	1.8	1.4
Glibenclamide	6.8	4.4	3.1	2.4	1.9
Glimepiride	7.6	5.3	3.5	1.8	1.1
ELSD					
Glipizide	8.8	9.9	11.8	8.0	6.4
Gliclazide	10.7	2.6	15.2	7.9	10.2
Glibenclamide	26.3	11.4	12.6	10.0	11.0
Glimepiride	8.9	8.7	14.3	11.6	11.7

value over 125% compared with UV detection and CAD. The % recovery of CAD was better than that of UV detection or ELSD, as seen in Table 1.

Relative standard deviation (RSD) was used to express the precision. From the results listed in Table 2, we can conclude that both UV detection and CAD are much more precise than ELSD. Although CAD and ELSD share the same aerosol formation mechanism, ELSD showed less precision than CAD in most cases, especially at lower concentrations. All of these results reflected the differences in the detection methods [10]. In CAD, aerosol particles are detected by electrical aerosol detection technology, not by light scattering as in ELSD. In this way, CAD can be more sensitive, even though the size of analytes might not be uniform. Although the RSD of CAD is somewhat worse than that of UV detection at low concentrations, we found that the precision of CAD was comparable with that of UV detection at higher concentrations such as 700 and 900 ng.

3.4. Limit of detection

In order to compare the sensitivity of UV detection, ELSD and CAD, the limit of detection (LOD) was evaluated. According to the results listed in Table 3, CAD exhibited the best sensitivity, with LOD of about 25, 16, 48 and 21 ng for glipizide, gliclazide, glibenclamide and glimepiride, respectively. The LOD values of CAD were similar with that of UV detection, but were much better than that of ELSD. Especially, for the detection of gliclazide, CAD was three times as good as UV detection, and even five times as ELSD.

Table 3
Limit of detection (ng) for UV detection, CAD and ELSD.

	UV	CAD	ELSD
Glipizide	36	25	73
Gliclazide	47	16	76
Glibenclamide	45	48	76
Glimepiride	27	21	77

3.5. Commercial sample analysis

The proposed methods were applied to screen anti-diabetic drugs and dietary supplements obtained in the market of Korea, China and Canada. Normally, two or more active ingredients of anti-diabetic drugs are not present in the same tablet or capsule formulation [30], however, for screening counterfeit drugs, or dietary supplements that may contain anti-diabetic drugs, our methods would be applicable. As shown in Figs. 3–5, four kinds of anti-diabetic drug tablets and three kinds of dietary supplements were selected as target samples. No interference peaks existed to disturb the detection of active components. Neither counterfeit drugs nor the illegal adulterants were found in the samples. Similar to the results using the standards, CAD and UV were much more sensitive than ELSD although CAD still gave the best sensitivity for the screening of gliclazide.

4. Conclusions

A comparison of three kinds of detectors (UV detection, ELSD and CAD) used in the HPLC system was undertaken. Four UV-absorbing anti-diabetic drugs were simultaneously detected by UV detection, ELSD and CAD in order to evaluate the performance parameters of those detection methods—linearity, accuracy, precision and limit of detection. In the present study, CAD was found to be superior to both ELSD and UV detection for the analysis of 4 anti-diabetic drugs (glipizide, gliclazide, glibenclamide and glimepiride). Therefore, thanks to its superior performance, ease of use, comparatively low cost and notably uniform responses, CAD has the potential to be the universal detector of choice for the determination of anti-diabetic drugs, and promises further application in pharmaceutical analysis as well.

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