# Analysis of Counterfeit Antidiabetic Drugs by UHPLC with the Agilent 1220 Infinity Mobile LC

## Application Note

Small Molecule Pharmaceuticals & Generics

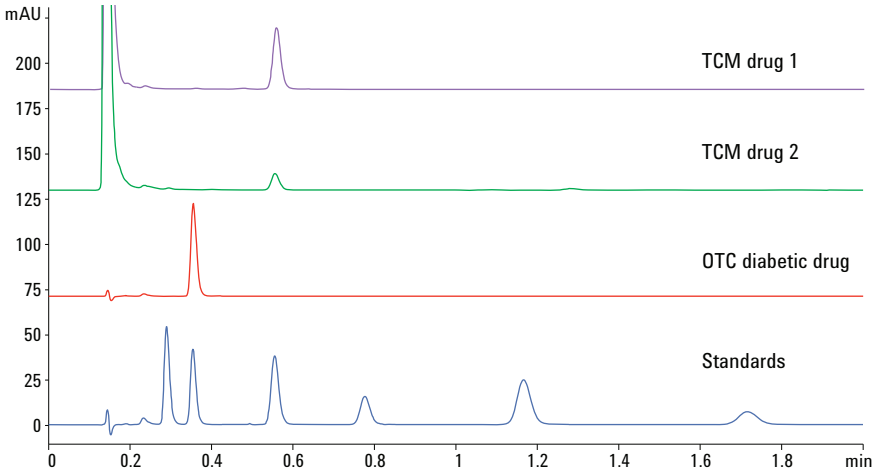

### Author

Sonja Schneider
Agilent Technologies, Inc.
Waldbronn, Germany



### Abstract

Counterfeiting in the pharmaceutical industry has become a serious and underreported problem, particularly in developing countries. This Application Note shows the analysis of antidiabetic drugs of the sulfonylurea class using the Agilent 1220 Infinity Gradient LC system with diode array detector (DAD). Both HPLC and UHPLC methods revealed excellent precision, accuracy and linearity as well as comparable limits of detection and quantification. With retention time and spectral confirmation, chemical antidiabetic substances could be identified and quantified in antidiabetic drugs and dietary supplements.


Agilent Technologies

## Introduction

The production of counterfeit drugs is a huge problem in developing countries, especially in parts of Africa and Asia[1], where approximately 50% of all sold drugs are fake. According to the Food and Drug Administration (FDA), counterfeit drugs comprise approximately 10% of the global medicine market. Counterfeiting is found in both branded and generic drugs, containing the wrong amount or the wrong active ingredient[2]. Chemical drugs can be found in adulterated dietary supplements, where no chemical active ingredient is allowed.

This Application Note shows the analysis of six antidiabetic drugs of the sulfonylurea class using the 1220 Infinity Gradient LC system with DAD. Figure 1 displays the six sulfonylurea drugs: glipizide, gliclazide, glibenclamide, glimepiride, gliquidone, and repaglinide.

These sulfonylurea drugs are commonly used in clinics to treat type II diabetes mellitus patients. The drugs stimulate the pancreas to release insulin, which lowers blood sugar. Approximately 90% of diabetes patients suffer from type II diabetes, which represents noninsulin dependent diabetes mellitus[2]. The analysis of type II antidiabetes drugs is very important to ensure their safety and efficacy.

The 1220 Infinity Gradient LC system with DAD enables the analysis of potentially counterfeit and adulterated antidiabetic drugs and dietary supplements to quantify and identify the active chemical ingredients.

The Agilent 1220 Infinity Mobile LC Solution is a robust and rugged system for on-site measurement because it is resistant against shocks or vibrations during transportation. The 1220 Infinity Mobile LC Solution can be used in a mobile laboratory as for on-site drug analysis.

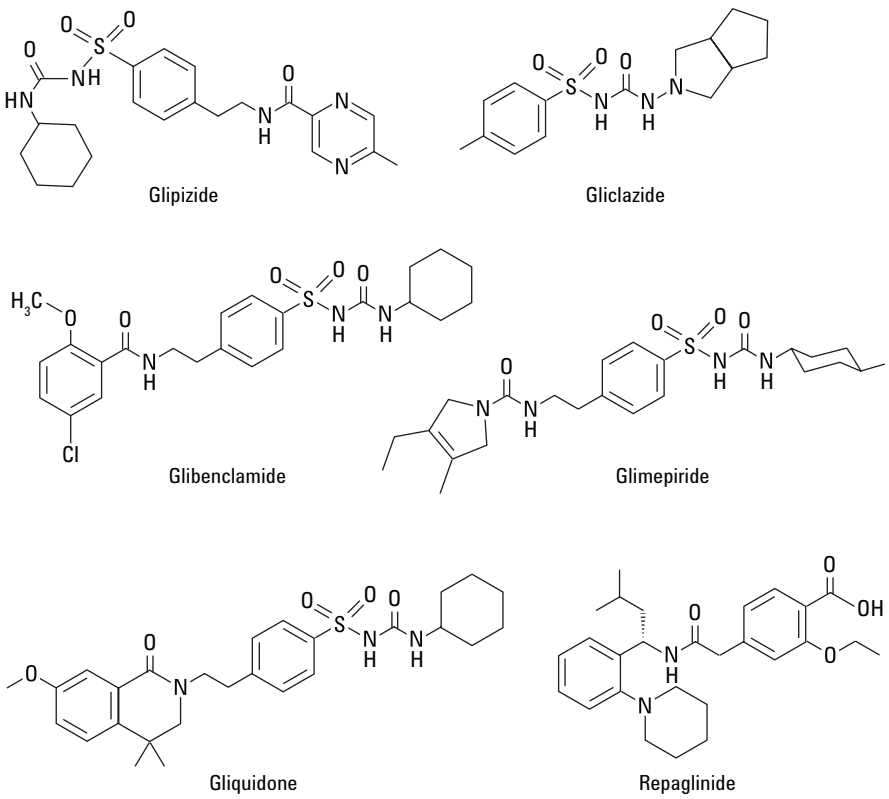



Figure 1. Chemical antidiabetic drug substances of the sulfonylurea class.

## Experimental

The Agilent 1220 Infinity Gradient LC system with DAD (G4294B) was equipped with a dual gradient pump with integrated degasser, autosampler, column compartment, and the diode array detector. For transportation, the LC can be mounted on a transportation plate, 1220 Infinity Mobile Upgrade Kit (G4292A).

### Sample

Antidiabetics standards of glipizide, gliclazide, glibenclamide, glimepiride, and repaglinide and the excipients silica, starch, soluble, talc, magnesium stearate, cellulose, polyvinylpyrrolidone, and hypromellose were purchased from Sigma-Aldrich, St. Louis, MO, USA. Gliquidone was purchased from LGC Standards, Teddington, UK. The antidiabetic drug and the dietary supplements were obtained through online pharmacies in China.

Stock solutions of antidiabetics were prepared at a concentration of 1 mg/mL in methanol. The standard solutions of 100 µg/mL were prepared from the stock solutions. The excipient mixture and the samples were filtered using Agilent Captiva Premium Syringe Filter, regenerated cellulose membrane, 15 mm diameter, 0.45 µm pore size (p/n 5190-5109).

All solvents were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak).

### Columns

- Agilent ZORBAX Eclipse Plus C18 4.6 × 150 mm, 5 µm (p/n 959993-902)
- Agilent ZORBAX RRHD Eclipse Plus C18, 3 × 50 mm, 1.8 µm (p/n 959757-302)

### Software

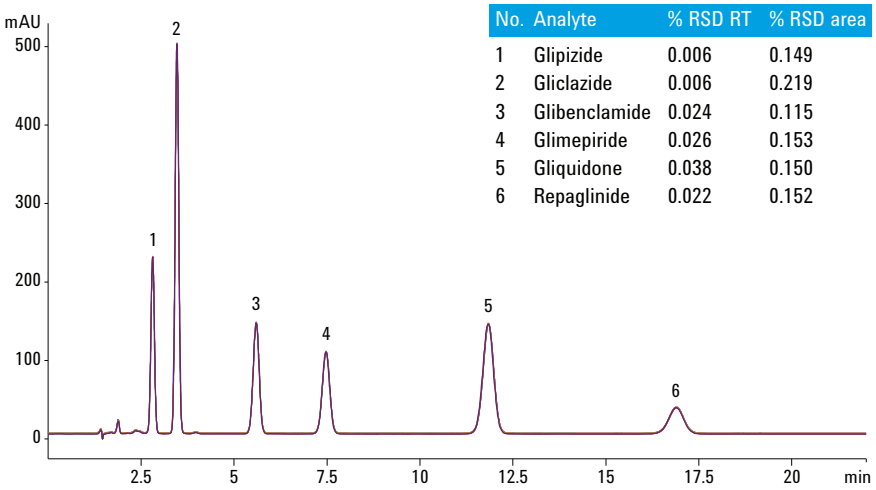
- Agilent OpenLAB CDS ChemStation Edition for LC & LC MS Systems, Rev. C.01.04 [35]
- Agilent OpenLAB CDS 3D UV Add-On software

## Results and Discussion

The antidiabetic drug standards were analyzed under HPLC conditions according to Yao *et al.*[2], see Figure 2. Six consecutive runs were analyzed for their precision regarding retention time and area. The relative standards deviation (RSD) of retention time and area was found to be excellent, below 0.04% and 0.22% respectively.

### Chromatographic Conditions

| | 4.6 × 150 mm, 5 µm | 3 × 50 mm, 1.8 µm |
|---|---|---|
| Solvent | Methanol:10 mM phosphate buffer, pH 3 (70:30) | Methanol:10 mM phosphate buffer, pH 3 (65:35) |
| Flow rate | 1 mL/min | 1.5 mL/min |
| Isocratic | Stop time – 25 minutes | Stop time – 2 minutes |
| Injection volume | 10 µL | 1.4 µL |
| Temperature TCC | RT | 60 °C |
| DAD | 230 nm/16 nm Ref.: off | 230 nm/16 nm Ref.: off |
| Peak width | > 0.025 minutes (0.5 second response time) (10 Hz) | 0.0063 minutes (0.13 second response time) (40 Hz) |




| No. | Analyte | % RSD RT | % RSD area |
|---|---|---|---|
| 1 | Glipizide | 0.006 | 0.149 |
| 2 | Gliclazide | 0.006 | 0.219 |
| 3 | Glibenclamide | 0.024 | 0.115 |
| 4 | Glimepiride | 0.026 | 0.153 |
| 5 | Gliquidone | 0.038 | 0.150 |
| 6 | Repaglinide | 0.022 | 0.152 |

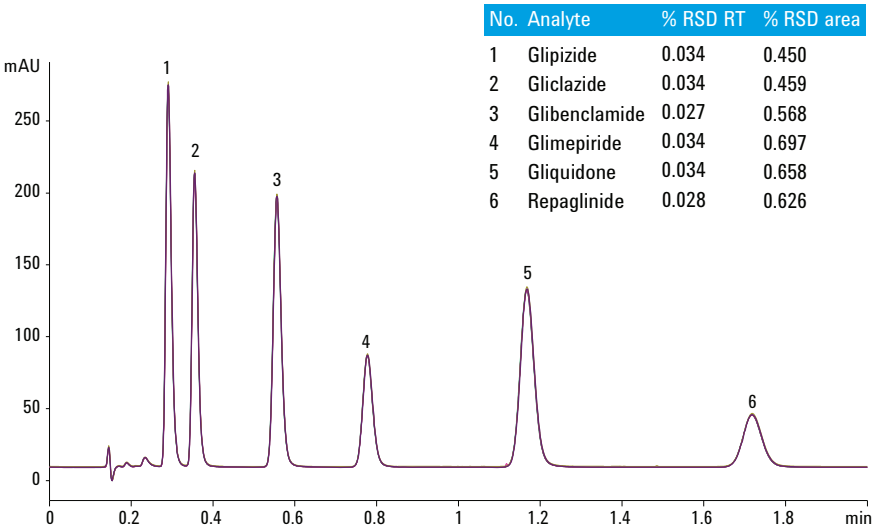
Figure 2. Six consecutive runs of six antidiabetic drug standards on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm.

To shorten the analysis time of the six antidiabetic drug standards, the method was transferred to an UHPLC method using an Agilent ZORBAX RRHD Eclipse Plus C18, 3 × 50 mm, 1.8 µm column. In comparison to a 4.6-mm id column, it was also possible to save solvent (over 85% per analysis) using lower flow rates with the 3-mm column, but gaining the same results. Figure 3 displays the UHPLC analysis together with the precision results for retention time and area.

The RSD of retention time and area was found to be below 0.04% respectively 0.7% for six consecutive runs. Regarding the over 10x shortening of the analysis time, the RSDs of the UHPLC method were still excellent.

Both methods were evaluated regarding linearity and limit of detection (LOD) and limit of quantitation (LOQ). Seven different concentration levels (from 100 µg/mL down to 0.14 µg/mL, 1:3 dilution) were prepared from the stock solutions and the linear relationship was determined between the peak area and the corresponding concentrations. LOD and LOQ were defined as the signal-to-noise ratio of 3:1 respectively 10:1. Table 1 shows the results of the evaluation. Both methods showed very high linearity with coefficients of determination ($R^2$) of 0.9999 for UHPLC and 1 for HPLC. LOD and LOQ were improved using the UHPLC conditions, double, for example, for glibenclamide.

Both methods were examined for interference of seven of the most common excipients. Due to the hydrophilic properties of the excipient substances, resulting in a very early elution, none of the excipients showed any interference (see Table 2).




| No. | Analyte | % RSD RT | % RSD area |
|---|---|---|---|
| 1 | Glipizide | 0.034 | 0.450 |
| 2 | Gliclazide | 0.034 | 0.459 |
| 3 | Glibenclamide | 0.027 | 0.568 |
| 4 | Glimepiride | 0.034 | 0.697 |
| 5 | Gliquidone | 0.034 | 0.658 |
| 6 | Repaglinide | 0.028 | 0.626 |

Figure 3. Six consecutive runs of six antidiabetic drugs standards using an Agilent ZORBAX RRHD Eclipse Plus C18, 3 × 50 mm, 1.8 µm.

Table 1. Linearity and LOD/LOQ, a comparison between HPLC and UHPLC conditions.

| Analyte | HPLC $R^2$ | UHPLC $R^2$ | HPLC LOD (pg) | UHPLC LOD (pg) | HPLC LOQ (pg) | UHPLC LOQ (pg) |
|---|---|---|---|---|---|---|
| Glipizide | 1 | 0.9999 | 66 | 38 | 221 | 127 |
| Gliclazide | 1 | 0.9999 | 76 | 46 | 254 | 153 |
| Glibenclamide | 1 | 0.9999 | 96 | 44 | 319 | 148 |
| Glimepiride | 1 | 0.9999 | 121 | 80 | 403 | 268 |
| Gliquidone | 1 | 0.9999 | 147 | 67 | 490 | 222 |
| Repaglinide | 1 | 0.9999 | 469 | 214 | 1563 | 714 |

Table 2. Interference of seven tested excipients.

| Excipient | Interference |
|---|---|
| Silica | - |
| Starch, soluble | - |
| Talc | - |
| Magnesium stearate | - |
| Cellulose | - |
| Polyvinylpyrrolidone | - |
| Hypromellose | - |

Both HPLC and UHPLC methods were evaluated for their precision and accuracy regarding the calculated amount of the six antidiabetic drug substances. For this experiment, 50 µg/µL per antidiabetic substance was spiked into an excipient mixture, dissolved in methanol. Three replicates were analyzed on three consecutive days to determine the accuracy as well as the intra and interday precision. Table 3 displays the comparison of accuracy and precision between the HPLC and UHPLC method. Accuracy was comparable between the HPLC and UHPLC method. Due to the shortening of the UHPLC method, the intra and interday precision was slightly better for the HPLC method. However, excellent precision was gained with both methods.

One antidiabetic over-the-counter (OTC) drug sample (OTC diabetic drug) and two dietary supplements from traditional Chinese medicine (TCM drug 1, TCM drug 2) were analyzed for the content of chemical antidiabetic drug substance. Figure 4 shows the chromatograms obtained from the samples compared to the standard mixture. The asterisks mark peaks, resulting from the other ingredients of the capsules (main ingredient: bitter melon and other natural ingredients).

Table 3. Precision and accuracy amount - HPLC versus UHPLC.

| Analyte | Spiked concentration (µg/mL) | HPLC accuracy (%) | UHPLC accuracy (%) | HPLC intraday precision (%) | UHPLC intraday precision (%) | HPLC interday precision (%) | UHPLC interday precision (%) |
|---|---|---|---|---|---|---|---|
| Glipizide | 50 | 102.35 | 99.83 | 0.13 | 0.73 | 0.17 | 0.83 |
| Gliclacide | 50 | 99.09 | 100.79 | 0.04 | 1.13 | 0.75 | 1.24 |
| Glibenclamide | 50 | 98.35 | 101.55 | 0.03 | 0.85 | 0.21 | 0.94 |
| Glimepiride | 50 | 96.84 | 101.61 | 0.05 | 0.26 | 0.42 | 0.81 |
| Gliquidone | 50 | 98.79 | 100.20 | 0.07 | 0.32 | 0.42 | 0.92 |
| Repaglinide | 50 | 97.97 | 104.56 | 0.11 | 0.15 | 0.49 | 0.97 |

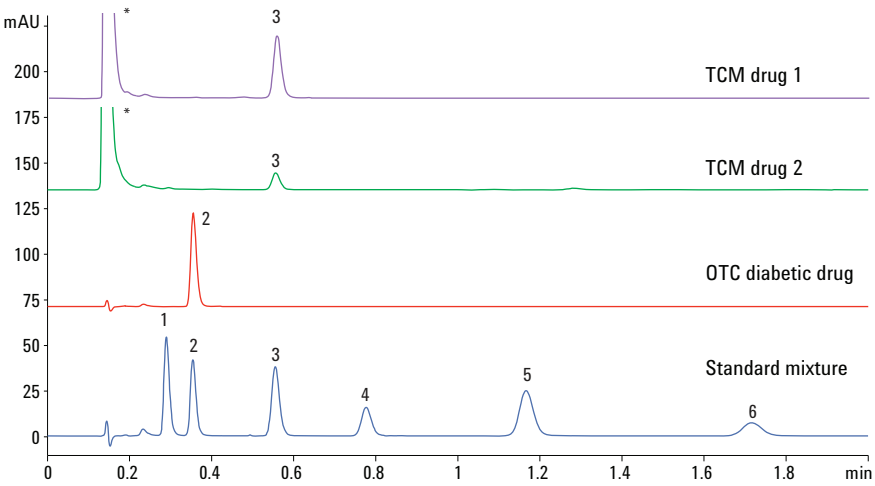



Figure 4. Analysis of antidiabetic drug and dietary supplements.

In addition to identification through retention time, the occurrence of the sulfonylurea antidiabetes drugs was authenticated with spectral confirmation using an UV-library, which was generated with the standards, dissolved in methanol. Gliclazide, as well as glibenclamide, was identified with high match factor in the samples using the UV-library. Figure 5 shows overlays of spectra from standards with spectra generated from the drug samples.

The amount of gliclazide in the OTC diabetic drug was 93.75%. Both dietary supplements from TCM, which should not contain any chemically active antidiabetes drug, revealed the presence of glibenclamide as active sulfonylurea drug, see Table 4.

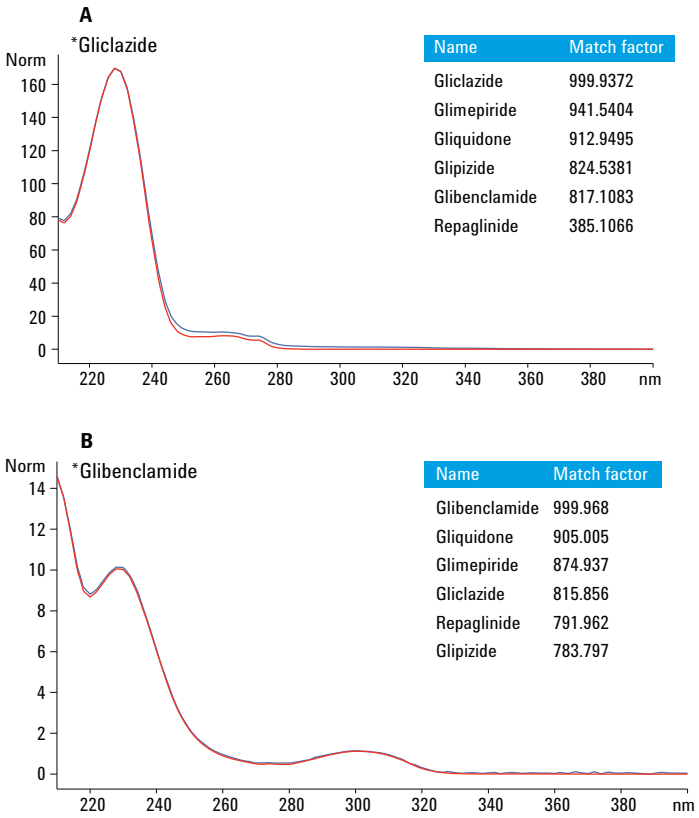



Figure 5. Spectral confirmation of chemical antidiabetes substances in antidiabetes drugs (A) and dietary supplements (B).

Table 4. Analysis of antidiabetic drugs and dietary supplements on their content of chemical antidiabetic drugs.

| | Chemical antidiabetic drug | Found content |
|---|---|---|
| OTC diabetic drug | Gliclazide 80 mg/tablet | 93.75% |
| TCM drug 2 | No chemical antidiabetic drug allowed | 0.94 mg/capsule |
| TCM drug 1 | No chemical antidiabetic drug allowed | 3.44 mg/capsule |

## Conclusion

The analysis of six sulfonylurea antidiabetic drugs was shown with both HPLC and UHPLC methods using the Agilent 1220 Infinity Gradient LC system with DAD. Both methods revealed excellent retention time and area precision as well as excellent linearity. LOD and LOQ were comparable (slightly better for UHPLC). Both were found to be in the two-digit and three-digit pg range, respectively, on column. The evaluation of accuracy showed high accuracy with values approaching 100%. The intra and interday precision was found to be excellent for both methods.

The samples of antidiabetic drugs and TCM dietary supplements were tested for their content of active chemical antidiabetes substance. In both TCM dietary supplements, an active chemical antidiabetes ingredient (glibenclamide) was found. Glibenclamide is not supposed to be in the capsules and is not marked on the capsule box as an ingredient. The chemical antidiabetic drug was identified using spectral confirmation with a UV-library in addition to the identification through retention time.

The drug analysis can be carried out on-site in a mobile laboratory using the Agilent 1220 Infinity Mobile LC Solution due to its resistance against shocks or vibrations during transportation.

The 1220 Infinity Gradient LC system with DAD is perfectly suited for the analysis of antidiabetic drugs or dietary supplements to identify and quantify the active chemical substance to detect counterfeit drugs or adulteration of dietary supplements.

## References


1. Cockburn *et al*., "The Global Threat of Counterfeit Drugs: Why Industry and Governments Must Communicate the Dangers", PLoS Med 4(9): e289. doi:10.1371, **2005.**

2. Yao *et al*., "Development of a RP-HPLC method for screening potentially counterfeit antidiabetic drugs", *Journal of Chromatography B,* Volume 853, 254-259, **2007**.

www.agilent.com/chem/1220


Published in the USA, April 1, 2013
5991-2177EN

Agilent Technologies