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Short Communication

The Altered Efficacy of Traditional Antidiabetic Formulations in Chittagong Division: Metformin Admixing

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ABSTRACT

Traditional medicine describes the treatment of diseases using a traditional medicinal practitioner's plant-based prescriptions. Although most people in developing countries continue to rely on traditional medicinal practices, many practitioners add modern active pharmaceutical ingredients (APIs) to their formulations. The aim of this study was to investigate the inclusion of metformin, an antidiabetic API, in traditional formulations, which represents a departure from standard practice. Samples were collected based on reports obtained from a short survey of practitioners in Chittagong City (Samples A, B, C, E, and F) and Rangamati District (Samples D, H, I, and J). The presence of metformin in the collected traditional formulations was confirmed using reversed-phase high-performance liquid chromatographic (HPLC) analysis comparing against a metformin standard. The HPLC chromatograms indicated the adulteration of the traditional medicine Samples D, E, I, and J with metformin, whereas the Samples A, B, C, F, and H were devoid of metformin admixture. The Samples D, E, I, and J contained 0.2169 ± 0.0018 ppm, 1.0714 ± 0.01 ppm, 2.8311 ± 0.01 ppm, and 0.0309 ± 0.003 ppm metformin, respectively. Inappropriate doses of metformin added to traditional medicines have been reported to result in detrimental health effects for patients. These results demonstrated the intentional use of metformin in traditional antidiabetic drugs by traditional practitioners to increase their credibility, which could represent a risk to the safety of patients who depend on traditional medicine.

Keywords: Traditional medicine, Metformin, High Performance Liquid Chromatography, Antidiabtic, Adulterant.

Introduction

Traditional healing practices represent essential and intrinsic components of healthcare systems in almost all countries worldwide. Almost 80% of the population in developing countries relies on traditional medicines.[1] The World Health Organization (WHO) states that traditional medicine is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness.[2]

Traditional medicines are typically prepared from plants and plant-based products and sometimes include components derived from small animals. Approximately 21,000 plant species are used for medicinal purposes globally. Over 7,500 species are estimated to be used by 4,365 ethnic communities in India for human and veterinary healthcare purposes, and approximately 2,500 plant species are being used for indigenous methods of treatment.[4] The individuals who practice traditional methods for the treatment and cure of diseases are known as traditional healers, traditional folk healers (TFH), traditional doctors, *Vaidya*, *Hakim*, or Amchi, among other names.[3] Traditional medicines are perceived to be safe, affordable, and associated with improved patient adherence compared with synthetic drugs.[5]

Therefore, the preparation of traditional medicines (TMs) consist of plant-derived substances[6], with no or minimal industrial processing or the use of active pharmaceutical ingredients (APIs), which can enhance the efficacy of the formulations and result in a number of complications. Unfortunately, many traditional practitioners admix APIs in their formulations, which are referred to as adulterations, to improve their credibility. Some patients use these formulations to achieve immediate healing, which is a major concern among health professionals.[7] Several studies have detected the presence of approved drugs and unapproved analogs in herbal medicines.[8,9] The presence of various synthetic drugs has been identified in herbal products marketed in Saudi Arabia, including tadalafil, sildenafil, and glibenclamide.[10] These types of drugs have been reported to cause a number of adverse effects in addition to transient benefits.

According to a survey performed in 2016 in Bangladesh, metformin was the most commonly prescribed antidiabetic drug, prescribed to 30.69% of all patients with diabetes, followed by glimepiride (10.9%), glipizide (5.45%), pioglitazone (1%), and other drugs.[11] Consequently, in this study, we analyzed the presence of metformin in samples of traditional diabetic medicines.

The International Diabetes Federation estimated that approximately 7.1 million people live with diabetes in Bangladesh, with an equal

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number of individuals who likely have undetected diabetes, and these numbers are expected to double by 2025. The prevalence of diabetes is expected to reach 13% by 2030, according to the International Diabetes Federation. Nearly 129,000 deaths were attributed to diabetes in Bangladesh in 2015, as reported by the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B).[12] In 2008, a survey conducted in Bangladesh indicated that among slum areas, 86% of women and 78% of men with diabetes either use inadequate or no medical treatments to control their diabetes. Even in non-slum areas, only 34% of men and women with diabetes receive adequate medical treatment.[13] Therefore, whether herbal remedies associated with the practices of traditional Bangladeshi medicine practitioners could offer safe, effective, and reasonable alternative therapies for diabetic patients should be explored.[14]

Therefore, identifying the incidence of adulterations in traditional antidiabetic medicinal formulations is highly significant for promoting awareness regarding this unethical and potentially harmful practice. In this study, we assessed the presence of adulterations in nine widely-consumed antidiabetic traditional formulations collected from different regions of greater Chittagong.[15] This study used a reversed-phase-high-performance liquid chromatographic technique to evaluate the antidiabetic potential of traditional formulations and the presence of the admixed antidiabetic API metformin.

Materials and Methods

Selection of Formulations and Locations

Traditional antidiabetic medicine formulations

Samples were collected from Chittagong Division from 15 May 2019. to 17 May 2019 (Table 1). A total of nine samples were obtained, including five samples from the Chittagong City area and four from Rangamati City. All obtained formulations were prescribed by renowned traditional practitioners from Chittagong City and Rangamati City (locally referred to as Baiddo). These formulations were collected according to the popularity of the natural healers. The obtained antidiabetic formulations are well known for their antidiabetic efficacy in the division.

Chemical and Reagents

All reagents and chemicals used in the study were of analytical grade. Metformin hydrochloride of pharmaceutical secondary standard (Sigma-Aldrich), sodium acetate trihydrate (AR Grade, Merck), acetonitrile (Scharlau), glacial acetic acid (AR Grade), and methanol (Sigma-Aldrich) were used.

Preparation of 20 MM Sodium Acetate Buffer

Sodium acetate trihydrate (2.72 g) was dissolved in 900 mL deionized water. The pH was adjusted to 4.0 with glacial acetic acid. The solution was mixed well and brought to a total volume of 1000 mL by the addition of deionized water.

Metformin Preparation

A stock solution of metformin (0.2 mg/mL) was prepared by dissolving metformin hydrochloride (2.56 mg, equivalent to 2 mg metformin) in 10 mL of the mobile phase. Metformin standards (1.25, 2.5, 5, and 10 mg/mL) were prepared by diluting the standard stock solution of metformin.

Sample Preparation

Samples of each of the nine formulations (25 mg, labeled A to J) were dissolved in 25 mL of 50% ethanol and filtered through 0.45 µm polytetrafluoroethylene syringe filter tips.

High-Performance Liquid Chromatography

A binary isocratic solvent system was used, in which the mobile phase consisted of 55% acetonitrile and 45% sodium acetate buffer. An Acclaim RSLC, C18, (2.1 × 100) mm, 2.2 µm column was used for this process. A photodiode array was used as the detector for a fixed 236 nm wavelength. The injection volume was 3 µL, and the flow rate was 0.2 mL/min. All nine traditional medicine formulation samples (Samples A to J) were analyzed by the system using the same conditions used for the metformin standard 236 nm. All samples were analyzed in triplicate.

Table 1: The areas and GPS locations of the areas the samples were collected from.

Sample ID	Areas of collection	GPS location
Sample A	Bakshir Bit, Chittagong	22.339135º, 91.840746º
Sample B	Bakshir Bit, Chittagong	22.339135º, 91.840746º
Sample C	Bakshir Bit, Chittagong	22.339368º, 91.841638º
Sample D	Vedbedi Bazar, Rangamati	22.654155º, 92.159358º
Sample H	Vedbedi Bazar, Rangamati	22.654155º, 92.159358º
Sample I	Vedbedi Bazar, Rangamati	22.654155º, 92.159358º
Sample F	Bakshir Bit, Chittagong	22.339376º, 91.842379º
Sample E	Bakshir Bt, Chittagong	22.339135º, 91.840746º
Sample J	Banarupa, Rangamati	22.657796º, 92.168663º

Statistical Analysis

Statistical analysis was performed and presented as the standard deviation and relative standard deviation. The relative standard deviation was calculated using the following formula.

$$\text{Relative Standard Deviation} = \frac{(\text{Standard Deviation})}{\text{Mean}} \times 100$$

Results and Discussion

Table 1 presents the areas from which the formulation samples were collected, including the GPS locations for the traditional medicinal practitioners. The organoleptic properties of the nine samples, including color and solubility parameters, are presented in Table 2. The formulations were solid in nature and were insoluble in water but could be solubilized in 50% alcohol. The metformin standard sample was analyzed by the HPLC system at 236 nm. The average retention time for various concentrations of metformin was 1.077 minutes. Figure 1 presents the calibration curve for the metformin standard, based on the analysis of five different concentrations: 1.25 ppm, 2.5 ppm, 5 ppm, 10 ppm, and 20 ppm. The metformin concentration, in ppm, was plotted against the peak area. The HPLC chromatogram for the metformin standard is shown in Figure 2. The chromatograms of the nine samples were compared against the retention time recorded for the metformin standard (Figures 2 and 3).

Each sample was independently analyzed three times. The chromatograms for the nine formulation samples (Sample A to J), as detected by the photodiode detector array, are shown in Figures 2 and 3. Chromatogram X in Figure 2 is the compilation of the chromatograms for four samples in which metformin was detected based on the comparison of the retention times compared with that for the metformin standard. Table 3 presents the quantification of metformin concentrations detected in Samples D, E, I, and J, showing the detection of 0.2169 ± 0.0018 ppm, 1.0714 ± 0.01 ppm, 2.8311 ± 0.01 ppm, and 0.0309 ± 0.003 ppm metformin, respectively, associated with retention times of 1.00 min, 1.00 min, 1.17 min, and 1.15 min, which can be directly compared with the retention time of the metformin standard. A previous study revealed that the adulteration of Chinese herbal medicines with synthetic drugs was a potentially serious problem that should be addressed by the implementation of adequate regulatory measures.[16] Thus, the quality and composition of TMs, which are 'manufactured' by local nonprofessional practitioners, must be examined carefully. For the

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purposes of this study, we assayed the nine samples of traditional medicinal formulations to determine whether any of them were adulterated with metformin. Briefly, the presence of metformin was detected in sample E, collected from Chittagong City, and Samples D, I, and J, collected from Rangamati District. These four samples presented similar retention times to that for the metformin standard. The presence of metformin was not detected in the remaining five samples. The other unnecessary peaks observed in the chromatograms might reflect the presence of contamination or be caused by variations in the analytical conditions, such as temperature. In conclusion, four of nine samples showed the presence of metformin, indicating the occurrence of adulteration by modern medicines in traditional medicinal formulations, which should be regulated through the enactment and enforcement of laws. Metformin was used at 1.25 ppm in a 3.0 µL sample volume, analyzed using a 236-nanometer wavelength, The metformin program run time was 5 minutes, and the average retention time was 1.077 minutes. HPLC chromatograms for four herbal samples (Samples D, E, I, and J) containing admixed metformin. The samples were analyzed in a binary isocratic solvent system consisting of 55% acetonitrile and 45% sodium acetate buffer using an Acclaim RSLC, C18 (2.1 × 100) mm, 2.2 µm HPLC column. The samples were eluted at retention times resembling that recorded for the metformin standard. Chromatogram X represents the combination of all original chromatograms (Figure 2).

Metformin was used at 1.25 ppm in a 3.0 µL sample volume, analyzed using a 236-nanometer wavelength. The metformin program run time was 5 minutes, and the average retention time was 1.077 minutes. HPLC chromatograms for five herbal samples (Samples A, B, C, F, and H) that did not contain admixed metformin. The samples were analyzed in a binary isocratic solvent system consisting of 55% acetonitrile and 45% sodium acetate buffer using an Acclaim RSLC, C18 (2.1 × 100) mm, 2.2 µm HPLC column. The samples did not elute at retention times resembling that for the metformin standard (Figure 3)

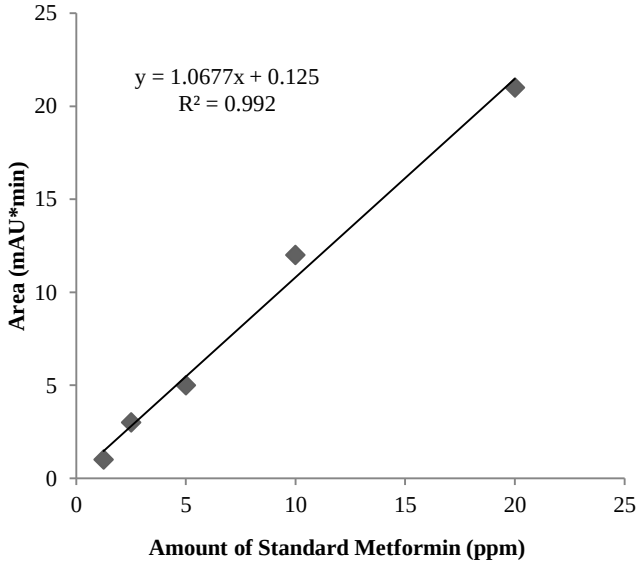

Figure 1: Calibration curve for the metformin standard. Samples were analyzed five times using increasing concentrations. The x-axis represents the metformin concentration in parts per million, and the y-axis displays the area of the HPLC chromatograms, in mAU·min. The metformin program run time was 5 minutes, and the average retention time was 1.077 minutes.

Table 2: Organoleptic properties of the samples

Sample	Water	50% Ethanol	Formulation Type	Color
A	Insoluble	Soluble	Solid (Coarse Powder)	Deep Brown
B	Insoluble	Soluble	Solid (Coarse Powder)	Deep Brown
C	Insoluble	Soluble	Solid (Coarse Powder)	Deep Brown
D	Insoluble	Soluble	Solid (Fine Powder)	Light Brown
E	Insoluble	Soluble	Solid (Coarse Powder)	Deep Brown
F	Insoluble	Soluble	Solid (Coarse Powder)	Deep Brown
H	Insoluble	Soluble	Solid (Bori)	Blackish
I	Insoluble	Soluble	Solid (Fine Powder)	Blackish
J	Insoluble	Soluble	Solid (Bori)	Blackish

Conclusion

This research explored a major gap in traditional medicinal manufacturing, exploring whether generic metformin was being used in traditional antidiabetic formulations. The long-cherished confidence in plant-based TMs has been partially distorted through this research. The promise of quick relief has recently attracted users of TMs, and the unethical practice of admixing generic APIs has increased. The practice of combining modern medicine with traditional medicine by traditional practitioners should be strongly discouraged. The government should address this issue and regulate the production of traditional formulations by requiring that natural products include appropriate labeling and packaging. The scientific evaluation of traditional formulations by a governmental regulatory body is necessary to maintain the quality and efficacy of traditional medicinal products. Antidiabetic traditional medicinal formulations are thought to contain a variety of unidentified antidiabetic compounds that can be explored by researchers for the future development of antidiabetic therapies.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 3: Quantification of metformin in four samples

Sample Name	Retention Time (min)	RSD (%)	Area (mAU* min)	RSD (%)	Height (mAU)	RSD (%)	Rel. Area (%)	RSD (%)	Amount (ppm)	RSD (%)	Plates	RSD (%)	Asymmetry	RSD (%)
A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D	1.00 ± 0.001	0.06	0.2349 ± 0.002	0.77	3.1226 ± 0.03	0.96	83.97 ± 0.15	0.18	0.2169 ± 0.0018	0.83	11.64 ± 0.2	1.72	2.87 ± 0.03	1.05
E	1.00 ± 0.001	0.06	1.1601 ± 0.002	0.20	12.2471 ± 0.04	0.33	58.51 ± 0.15	0.26	1.0714 ± 0.01	0.93	821 ± 1.0	0.12	2.77 ± 0.015	0.55
F	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I	1.17 ± 0.010	0.85	3.0655 ± 0.045	1.47	33.7585 ± 0.2	0.59	9.99 ± 0.10	1.00	2.8311 ± 0.01	0.35	978± 1.52	0.16	1.17 ± 0.02	1.71
J	1.15 ± 0.010	0.87	0.0335 ± 0.003	0.90	0.3402 ± 0.002	0.65	4.8 ± 0.08	1.67	0.0309 ± 0.003	0.97	1023 ± 1.0	0.10	2.02 ± 0.03	1.49

* - denotes the absence of metformin in the labeled samples.

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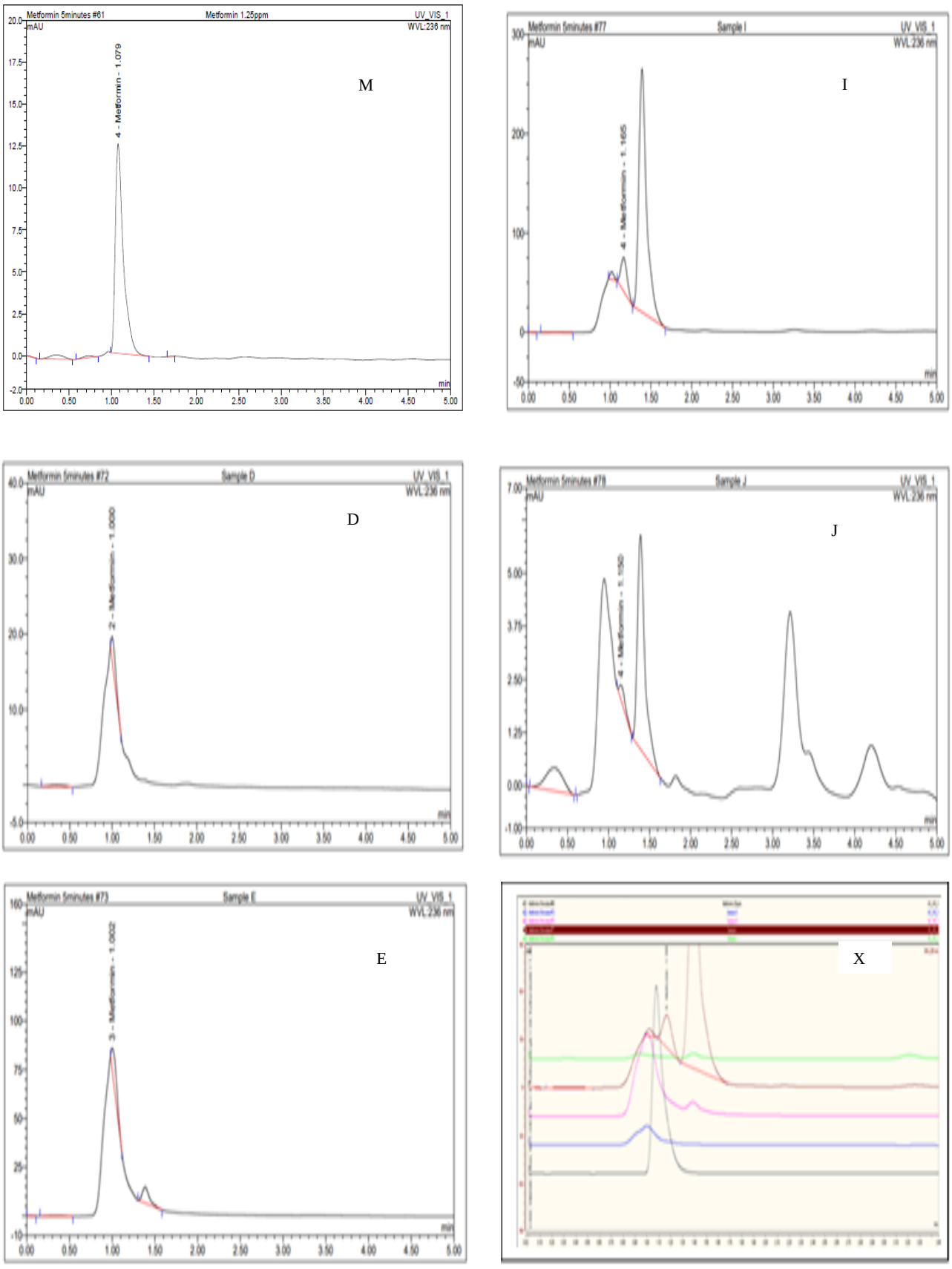

Figure 2: HPLC chromatogram for the HPLC-grade metformin standard (Metformin Standard, M).

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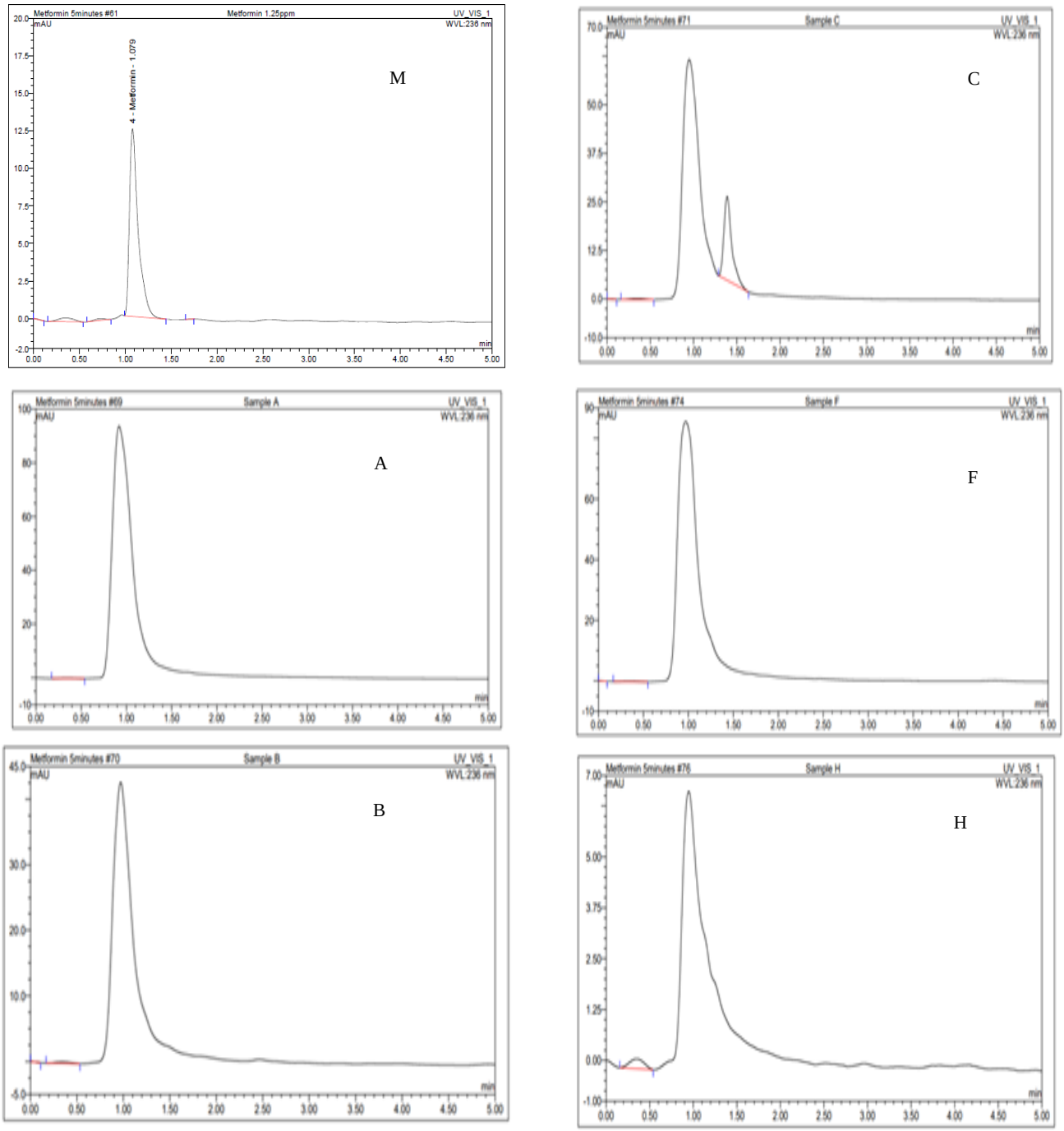

Figure 3: HPLC chromatogram for the HPLC-grade metformin standard (Metformin Standard, M)

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