ISSN: 2634 - 680X

Journal of Clinical Case Studies Reviews & Reports

SCIENTIFIC Research and Community

Research Article

Open Access

Evaluating Chromatographic and Spectroscopic Techniques in Counterfeit Drug Identification

Mesheal AL Abdulrahman[1], Halia Faya Asiri[2], Moteap Alhaily[3], Khattam AL Atawi[4], Ghadah AL Suhaibani[5], Mohammed Massir Ayed Al Anzi[6] and Abdul Wali Ajlouni[7]*

[1]Forensic Medical Services Center, Ministry of Health, Riyadh, KSA

[2]Pharmacy Services Department, King Saud University Medical City, King Saud University, Riyadh, KSA

[3]Forensic Medical Services Center, Ministry of Health, Riyadh, KSA

[4]Pathology & Clinical Laboratory Medicine Administration, KFMC, Riyadh, KSA

[5]Presidency of the Saudi Royal Guard, Poisons Department, Riyadh, KSA

[6]Forensic Medical Services Center, Ministry of Health, Riyadh, KSA

[7]Consultant in Nuclear Security, Radiation Safety, and CBRN. ANFAS- RADIATION & CBRN, ANFAS Medical Co. Riyadh

ABSTRACT

Counterfeit pharmaceuticals present a serious global health risk, with sildenafil being among the most frequently falsified drugs. Rapid and accurate detection methods are essential to ensure patient safety, maintain public trust, and support regulatory enforcement. This study evaluates and compares the performance of five analytical techniques-UV-Vis spectrophotometry, GC-MS/MS, HPLC-PDA, LC-MS/MS, and UPLC-QTOF-in the detection and authentication of sildenafil in genuine, generic, and potentially counterfeit samples.

UV-Vis provided a cost-effective preliminary screening tool, showing consistent λ max values (~290 nm) but limited quantitative accuracy. GC-MS/MS offered strong selectivity but was restricted by volatility and thermal stability requirements. HPLC-PDA balanced speed and robustness for routine quality control, while LC-MS/MS achieved the highest sensitivity and specificity, allowing accurate detection and quantification with short retention times. UPLC-QTOF delivered high-resolution mass measurements and precise fragmentation patterns, enabling confident molecular identification.

The results highlight LC-MS/MS and UPLC-QTOF as the most effective confirmatory techniques, with UV-Vis and HPLC-PDA serving as valuable tools for initial screening and routine analysis. Integrating multiple analytical methods provides a comprehensive approach to combating counterfeit pharmaceuticals, enhancing regulatory capacity, and safeguarding public health.

***Corresponding author**

Abdul-Wali Ajlouni, Consultant in Nuclear Security, Radiation Safety, and CBRN. ANFAS- RADIATION & CBRN, ANFAS Medical Co. Riyadh, KSA, E-mail: awajlouni@hotmail.com

Received: September 16, 2025; **Accepted:** September 19, 2025; **Published:** October 10, 2025

Keywords: Counterfeit Drugs, Sildenafil, Drug Security, Pharmaceutical Forensics, Drug Authentication, Patient Safety, UV-Vis, GC-MS/MS, LC-MS/MS, LC-PDA and UPLC-Q TOF

Introduction

Counterfeit medicines pose a critical threat to public health, pharmaceutical supply chain integrity, and the global economy. These illicit products endanger patients' lives, undermine the credibility of legitimate manufacturers, and damage the reputation of national health systems. The problem is particularly severe in countries with weak regulatory frameworks, inadequate quality control systems, and limited access to affordable medicines. Under such conditions, Falsified, Substandard, and Counterfeit (SSFFC) medical products proliferate, often bypassing official inspection and control mechanisms [1,2].

Economic drivers-including high market prices, medicine shortages, and limited availability-further incentivize the manufacture and distribution of counterfeit pharmaceuticals. As counterfeiters employ increasingly sophisticated methods, detecting falsified products demands advanced analytical capabilities. A wide range of techniques, including chromatographic and spectroscopic

methods, have been applied to assess pharmaceutical authenticity, quantify active ingredients, and identify chemical fingerprints that distinguish genuine from falsified drugs [3,4].

This study focuses on sildenafil, a widely used pharmaceutical that is frequently targeted by counterfeiters. Five analytical methods—UV-Vis, GC-MS/MS, LC-MS/MS, LC-PDA, and UPLC-QTOF-were evaluated for their efficiency in detecting genuine and generic sildenafil samples, including potential falsified products. By comparing performance across these techniques, this research aims to provide a practical reference for law enforcement, regulatory agencies, and quality control laboratories seeking reliable methods to combat counterfeit pharmaceuticals.

Materials and Methods

In the following sections, detailed procedures for analysis of different sildenafil samples using different analytical techniques are presented.

Chemicals

Sildenafil and sildenafil-D3 (Toronto Research Chemicals Inc. Toronto, Canada). Distilled water: Purelap option-Q (produced in our laboratory with Mini Pak Express 40). Propranolol Hydrochloride (Hikma Pharmaceutical Portugal, SA, 1mg/ml). Monopotassium phosphate (KH2PO4). Ethyl acetate (EtA). (Sigma-Aldrich®, 99.5%). Helium gas (99.9995%). Methane gas. Argon gas. Formic acid. O-Phosphoric Acid, 85% (Certified ACS), Fisher Chemical, UK. O-Potassium Dihydrogen Phosphate extra pure (Mol.Wt.136.09 grams/mole, Sigma Aldrich, Germany. HPLC grade methanol, Sigma Aldrich, Germany. HPLC grade Acetonitrile, Sigma Aldrich, Germany.

Instruments and Equipment

The analysis of Sildenafil samples involved several instrumental techniques. The basic characteristics for the techniques used in this work are briefly outlined below:

UV-Vis Spectrophotometer

UV-Vis spectrophotometer (PerkinElmer Instruments, model Lambda 35, USA) with 0.5, 1, 2 and 4 nm variable slits.

GC-MS/MS

Gas Chromatography Tandem Mass Spectrometry Agilent 7890B GC equipped with a 7693 Autosampler and 7000C Series Triple quadrupole (QQQ) mass spectrometer detector, MSD system (Agilent, USA). An Agilent Ultra Inert GC column, HP-5MSUI, was used to provide a highly inert flow path into the detector. The Agilent Mass Hunter Workstation software B.07.00SP2 was used for data analysis.

HPLC-PDA (DAD)

The UPLC system used for the analyses of the samples consisted of an UPLC with PDA detector, with the following properties: Perkin Elmer HPLC-PDA. Perkin Elmer HPLC Series-200. Pump: Perkin Elmer Series-200. PDA (Photo-diode-Array): Perkin Elmer Flexor PDA. Column: Lichrosphere 100RP-C8, (5µm), Length: 250 mm, Diameter: 4mm. Working Station Software: Total Chrome workstation. version 6.3, Iris for spectral data processing: Analytical Electronic Balance Mettler model AE-166, Switzerland. Hanna PH Meter, Hi-8510-N, Hanna Instruments, Portugal. Mobile Phase: HPLC grade acetonitrile 60%: Potassium Dihydrogen Phosphate Buffer 0.01M, 40%, PH: 3.0. Reference wavelength 230nm. Sample injection volume: 20µl. Total Run Time: 10 minutes.

LC-MS/MS

Liquid chromatography tandem mass spectrometry measurements was used in this part, the work was accomplished using Waters® XevoTM TQ Mass Spectrometer, an advanced, bench top, tandem quadrupole mass spectrometer designed for ultrahigh performance LC/MS/MS applications. The Xevo TQ MS is a robust platform for quantitative LC/MS/MS, featuring high speed MRM (Multiple Reaction Monitoring), with enhanced full scan spectral acquisition modes providing additional qualitative LC/MS/MS capabilities. Unlike conventional tandem quadrupole mass spectrometers, the Xevo TQ MS utilizes unique T-WaveTM1 and Scan Wave TM enabled collision cell technology to provide a highly flexible analytical tool capable of supporting both quantitative and qualitative studies. MS: Waters Quattro premier with Mass Lynx 4.1 software.

Mass Analyzer

Two high resolution quadrupole analyzers (MS1/MS2), plus pre-filters to maximize resolution and transmission while preventing contamination of the main analyzers. Collision cell: T-Wave enabled for optimal MS/MS performance at high data acquisition rates; Scan Wave enabled for enhanced MS/MS spectral performance (product ion scanning).

UPLC-QTOF

The UPLC-Q-TOF-MS/MS system consisted of: Two Exion LC AD pumps and an Exion LC AD autosampler coupled with a high resolution X500 Q-TOF mass spectrometer (Sciex, USA). The SCIEX OS 1.0 software from Sciex (Sciex, USA) contains instrument control, data acquisition, data processing, and reporting functionality, all in the one package. Chromatographic separation was achieved on an Agilent SB-C18 RRHD column (100 mm×3.0 mm, 1.8 µm) (Agilent Technologies, USA). All centrifugations were performed on a Sigma 3 - 30 K refrigerated centrifuge (Sigma, Germany). Ultrasonic process was operated on a KQ - 300 GDV Thermostat Ultrasonic Instrument (Kunshan, China).

Samples

The study samples included six types of sildenafil tablets; each type composed of 3 samples. Table (1) below represents the specifications of the six types. The reference type was a pure sildenafil powder. Sildenafil- D3 (1 mg) was used as the internal standard (IS). Certified Reference Standards of sildenafil and sildenafil- D3, were obtained from Toronto Research Chemicals Inc. Toronto, Canada. All samples were purchased in sealed packages and transported to the Laboratory.

Table 1: The Specifications of the Six Types of Sildenafil

Name	Batch number	Expiry date	Origin
Silden 100mg	1803824	May 2020	Egypt
Viavag 100mg	0110218	Feb 2021	Egypt
Nizagara 100mg	NZR - 121	Aug 2021	India
Nizagara 100mg	NZR - 118	Aug 2021	India
Erecta 100mg	VHO153	May 2020	S. A
Viagra 100mg	B188203-d3	Jun 2021	USA
Sildenafil citrate 100mg	2-SHE-124-1	Jun 2022	Canada
Sildenafil D3 25mg	239-GHZ-77-1	Jun 2022	Canada

Methods

Calibration Curves and Linearity

Genuine sildenafil (Toronto Research Chemicals Inc. Toronto, Canada) was taken as standard samples, and the standard stock solution (ST) was prepared by dissolving Genuine sildenafil powder in methanol. The five standards were prepared at 2.5, 5, 10, 20, and 40 µg/ml levels. These celebration samples were prepared by dilution of the standard stock solution with acetonitrile or ethyl acetate according to instrument needs.

Correlation coefficient (R2) values, may vary from negative one to positive one, but should be 0.97-1.00 for the method to be considered linear [5, 6].

Internal Standard (IS)

Sildenafil-D3 was used as the IS. A solution of 1 mg/ml of IS was made up by adding 10 ml of HPLC grade Acetonitrile/ methanol to 10 mg of sildenafil- D3, followed by brief vortexing then sonication for 15 minutes and stored at -18 ±2ºC. Enough stock solution was made up so that it could be used for the whole validation process. Internal standard was spiked into samples at 100 µg/ml for all instrumentations and measurements.

Preparation of Exhibit Samples for Screening

Initially, 18 samples were used to be screened by different analytical techniques. Three samples were taken from each pack, each sample was composed from one tablet. Initially, each sample was weighed to obtain the total mass of each tablet. The samples were scraped, and then homogenized individually using a pestle and mortar for the tablet's contents. A Kimble tube was used to prevent splashing as the Eppendorf tubes proved too small.

The samples were then dissolved by adding10 mL of a HPLC grade methanol to 10 mg of the sample. The concentration of each sample was 1 mg/ml.

The solutions were vortexed, sonicated for 15 minutes and then centrifuged at 3500 rpm for 10 minutes. Following this, 100 µl of each 1 mg/ml sample solution was then diluted further with 800 or 850 µl HPLC grade ACN or ETA, beside 100 or 50 µl of Sildenafil-D3 IS solution or propranolol, to produce 10 µg/ml working solutions for injection into the instrument for the following different types of analysis:

UV-Vis Analysis

The working solutions, as well as, the working standard solution, were divided in three parts, one dissolved in HPLC grade methanol, the second in 2.0 N HCl, and the third in 0.5 N NaOH, and then measured by Lambda 35 UV-Visible spectrophotometer. All measurements were carried out at room temperature (25 ±2°C).

GC-MS/MS Analysis

Preparation of Exhibit Samples for Screening

100µl of the 1 mg/ml solution was injected in the vial. The injector was operated in spitless mode with an injection volume of 2.0µl. Helium (1 ml/min) was used as a carrier gas and argon (1.5 ml/min) as a collision gas. The injection port and the transfer line temperature were held at 250°C and 280°C, respectively. The column temperature was programmed as follows: the initial temperature was 250°C, ramping at 25˚C/min to 320°C, holding for 15 min. The MS was operated in electron ionization (EI) mode (70 eV) with ion source temperature 300°C. The multiple-reaction monitoring (MRM) mode was used to detect and quantify the compounds.

Development of an acquisition method involves the following steps: setting the inlet and injection parameters, entering GC acquisition parameters, creating acquisition method for finding precursor ions, acquiring precursor ion scan data, determining precursor ion masses, creating acquisition methods for finding product ion masses, acquiring scan data for finding product ions, determining the product ions, creating an MRM method, acquiring MRM data, creating a quantitative analysis batch, creating an MRM quantitative analysis method, and quantitating a batch of unknown samples.

HPLC-PAD Analysis

Preparation of Mobile Phase

- 1.3609 grams of o-potassium Dihydrogen phosphate (0.01M, Ph 3.0) was weighed on analytical electronic balance accurately, transferred to beaker containing approximately 800 ml of deionized water (18.2M. ohms), the beaker was placed on electrical stirrer to dissolve the salt, Ph.3.0 was adjusted with 1N O-phosphoric acid (85%). The solution was transferred to 1.00 L volumetric flask and volume 1.00 liter adjusted with deionized water.
- Preparation of Isocratic Mobile Phase 600 ml of HPLC grade acetonitrile was mixed with 400 ml of phosphate buffer (60%: 40%), precautionary left it overnight in closed HPLC reagent bottle for well mixing and degassing.

LC-MS/MS Analysis

Preparation of Mobile Phase

The mobile phase consisting of 500 µl formic acid in 250 ml water and 250 ml acetonitrile. In addition to mobile phase-associated factors, use of an efficient and robust analytical column, which can also allow high-throughput separations, was preferred to increase total versatility of the methods.

All sildenafil samples were detected in the multiple reaction monitoring modes (MRM) according the following tasks: entering acquisition parameters and acquire data, determining precursor ion masses, finding optimum fragmentor voltage for maximum response, determining product ion masses, and then finding optimum collision energy for MRM acquisition.

HPLC-QTOF Analysis

Chromatographic Conditions

Chromatographic separation was performed on a SB-C18 RRHD column of Agilent (100 mm × 3.0 mm, 1.8 mm). A binary mobile solvent was used: mobile solvent A was 45% deionized water acetate solution (adjusted pH to 3.4 with acetic acid), and mobile solvent B was 125% methanol. The mobile phase was delivered at a flow rate of 0.7 mL/min with a gradient elution profile. The gradient began at 25% B for 2min, and then linearly ramped to 55% B within 11min, then ramped to 90% B in 1 min and held at 90% B for 2.0 min, then the column was re-equilibrated at 25% B for 2min prior to the next injection. The autosampler tray temperature was set to 15 ºC, while the column temperature was 40 ºC. The injection volume was 5 µl.

Mass Spectrometry Conditions

The Q-TOF high-resolution mass spectrometry (HRMS) was equipped with a Turbo V™ ion source and the ESI+ mode was applied. The Q-TOF spectra was generated in product ion scan mode at collision energies (CE) of -10 V with CE spread of 0 V.

Results

To construct the calibration curve five standard solutions were prepared (10, 25, 50, 75, 100 µ/ml concentration). The best

absorbance values of these solutions were noted at 291 nm wavelength (λmax for Sildenafil). Data was collected and plotted in a chart to obtain the standard curve (Fig. 1). From this curve a correlation coefficient value (R2) of 0.994518 was found. The graph shows negligible intercept and is described by the correlation equation:

$$Y = a X + b$$

Where
Y is the absorbance of 1 cm layer,
a is the slope,
b is the intercept and
X is the concentration of the measured solution in $\mu g.ml^{-1}$

$$Y = 0.022291 X + 0.011887$$

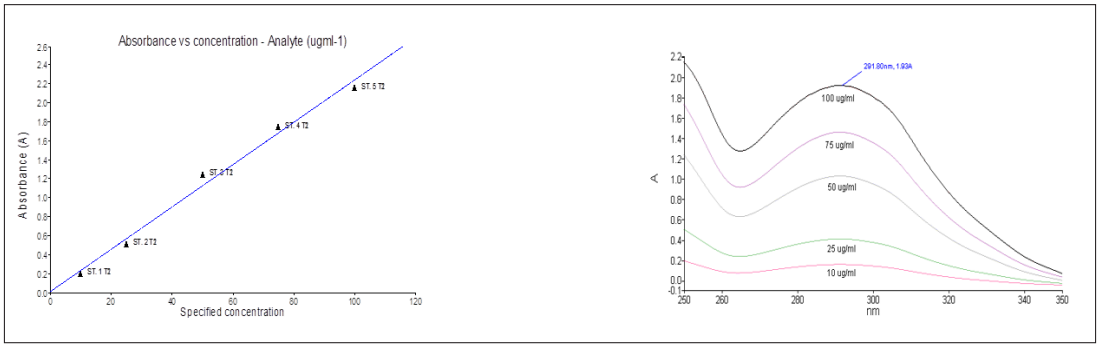

Figure 1: UV-VIS Spectra of Different Concentrations of Standard Sildenafil, and the Calibration Curve

UV-Vis Results for the Working Solution

The analysis of the working solutions and the working standard solution samples using Lambda 35 UV-Vis spectrophotometer, are presented in Fig. (1). Sildenafil absorption peaks, and absorbances, using methanol, HCL, and NaOH as solvents, are presented in Table (2).

Table 2: Absorption Peaks, and Absorbance using Different Solvents

Solvent	Wavelength				Absorbance			
	Sild	T1(PFZ)	T2(EPC)	T3(HI)	Sild	T1(PFZ)	T2(EPC)	T3(HI)
Methanol	291.38	291.41	291.52	291.64	0.76	0.83	0.78	1.28
HCL	286.49	286.46	287.58	287.81	0.49	0.36	0.79	1.15
NaOH	290.03	289.98	289.94	290.08	0.53	0.85	0.76	0.67

Part II: GC-MS/MS Results

Linearity

The linearity of the calibration curve was determined by analyzing reference standard solution containing Sildenafil at five different concentrations (2.5, 5, 10, 20, 40 µ/ml concentration). The internal standard solution (Sildenafil D3) was also prepared in Ethyl acetate. Internal standard was spiked into samples at 100ng ml-1

The calibration curve was plotted, shows good linearity in the studied working range, with correlation coefficient (R^2) greater than 0.9975. Fig. (2) shows the calibration curve. The correlation equation:

$$Y = a X + b$$

$$Y = 0.006834 X + 0.002848$$

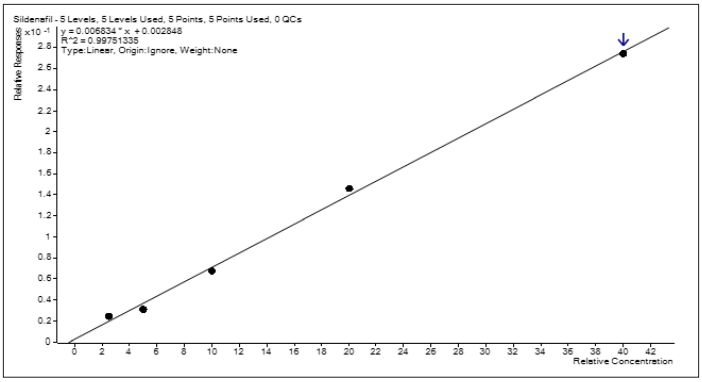

Figure 2: GC-MS/MS Calibration Curve for Sildenafil

GC-MS/MS Results for the Working Solution

The GC-MS/MS database development was carried out by creating a quantitative analysis method, which include the following steps: (1) scan for the precursor ions and then saved them to the method, (2) creation of acquisition data file, (3) determination the precursor ions masses for sildenafil in the acquired data file, (4) fragmentation the identified precursor ions masses and scanned for product ions, (5) creation of a sequence to acquire data for finding the product ions, (6) determination of the product ion for sildenafil in the specific acquired data file, (7) creation of an MRM method that finds sildenafil in a sample.

The optimized transitions were stored in optimizer data bases which contain three transitions per compound in addition to the conditions for the tested compounds. Table (3) shows the sildenafil names and the precursor ion and product ions for each and retention time of each. Fig. (3) shows the GC chromatograms and related MRM mass spectra of all sildenafil samples as they were analyzed in ethyl acetate solvent.

Table 3: MRM Transition Parameters of GC-MS/MS for the Determination of the Sildenafil Samples

No	Sample	Precursor Ion (m/z)	Product Ion (m/z)	Ret Time (min)
1	Sildenafil	478	101 61	13.077
2	T2(IND1)	478	101 61	13.067
3	T3(HI)	478	101 61	13.052
4	T4(IND2)	478	101 61	13.006
5	T5(EPC)	478	101 61	13.057
6	T6(JAM)	478	101 61	13.052
7	T7(PFZ)	478	101 61	13.032

Part III: HPLC- PDA Results

Linearity

HPLC-PDA method linearity was studied using different concentration levels of Sildenafil. The assay was linear over a concentration range of 2.5–40 µg/ml. The correlation coefficient value of the calibration curves (R2) was 0.9999. A correlation equation of

$Y = 0.1205X + 0.0504$

for the sildenafil standard samples was obtained. Each calibration point was measured three times. The acceptance criteria $R2 \geq 0.99$ concerning linearity were met for Sildenafil.

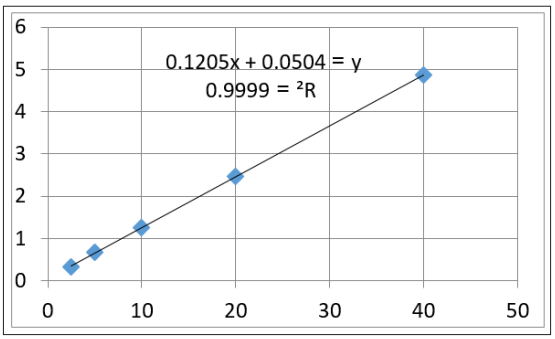

Figure 3: HPLC-PDA Calibration Curve for Sildenafil

HPLC-PDA Results for the Working Solution

HPLC-PDA method used for the determination of Sildenafil samples. The conditions of HPLC separation were optimized for the mobile phase composition by gradient elution. The experiments were carried out in order to select the most suitable gradient preparation of the mobile phase to obtain the best separation of Sildenafil.

Table (4) shows the retention time of each sildenafil sample. Figure. (3) shows the HPLC-PDA chromatograms of all sildenafil samples.

Table 4: The retention characteristics of different Sildenafil samples of HPLC-PDA methods

No.	Sample	Ret Time(min)
1	Sildenafil	5.03
2	T2(IND1)	5.08
3	T3(HI)	5.04
4	T4(IND2)	5.10
5	T5(EPC)	5.05
6	T6(JAM)	5.07
7	T7(PFZ)	5.07

Part IV: LC-MS/MS Results

Linearity

HPLC-MS/MS method linearity was studied using different concentration levels of Sildenafil. The assay was linear over a concentration range of 2.5–40 µg/ml. The correlation coefficient value of the calibration curves (R^2) was 0.9978. A correlation equation of

$$Y = 0.485922X + 1.15744$$

for the sildenafil standard was obtained. Each calibration point was measured three times. The acceptance criteria $R^2 \geq 0.99$ concerning linearity were met for Sildenafil.

Sildenafil standard samples gave perfect straight line at concentrations between 2.5 µg/mL and 40 µg/mL.

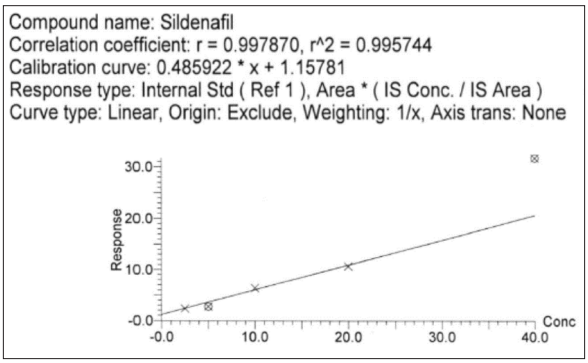

Figure 4: LC-MS/MS Calibration Curve for Sildenafil

LC-MS/MS Results for the Working Solution

The obtained MRM transition parameters of LC-MS/MS for the determination of the above-mentioned sildenafil samples are summarized in Table (5) The precursor ions (Q1) were obtained from the exact masses of each protonated or deprotonated molecular ion as [M + H]+ or [M − H]−. Two product ions (Q3) of each sample were selected among the fragmented ions from Q1 based on their high intensities. Two of the Q3 ions having the highest intensity was selected as the ion to be used for quantification.

Table 5: MRM Transition Parameters of LC-MS/MS for the Determination of the Sildenafil Samples

No	Sample	Precursor Ion (m/z)	Product Ion (m/z)	Ret Time (min)
1	Sildenafil	475.1	283.1 99.1	0.58
2	T2(IND1)	475.1	283.1 99.1	0.58
3	T3(HI)	475.1	283.1 99.1	0.58
4	T4(IND2)	475.1	283.1 99.1	0.58
5	T5(EPC)	475.1	283.1 99.1	0.58
6	T6(JAM)	475.1	283.1 99.1	0.58
7	T7(PFZ)	475.1	283.1 99.1	0.5

Part V: UHPLC-QTOF Results

Linearity

Calibration curve was constructed by plotting the analyte peak area (Y-axis) vs a series of analyte concentrations (X-axis). The correlation equation was described as

$$Y = a X + b,$$

which was used to calculate the concentrations of standards and samples. The linear relationship between the chromatographic peak area and the concentration of the analytes was investigated. The linearity of calibration curve was assessed by the correlation coefficient (R2). To construct the calibration curve seven standard solutions were prepared (5, 10, 20, 40, 80, 160, 320 µ/ml concentration). From this curve a correlation coefficient value (R2) was found, and the curve is described by the correlation equation:

$$Y = 3.46990\times10^{4}\, X + 4.76426\times10^{4}$$

As can be seen in Fig. (25) below, the calibration curve showed good linearity with R^2 value equals 0.99777.

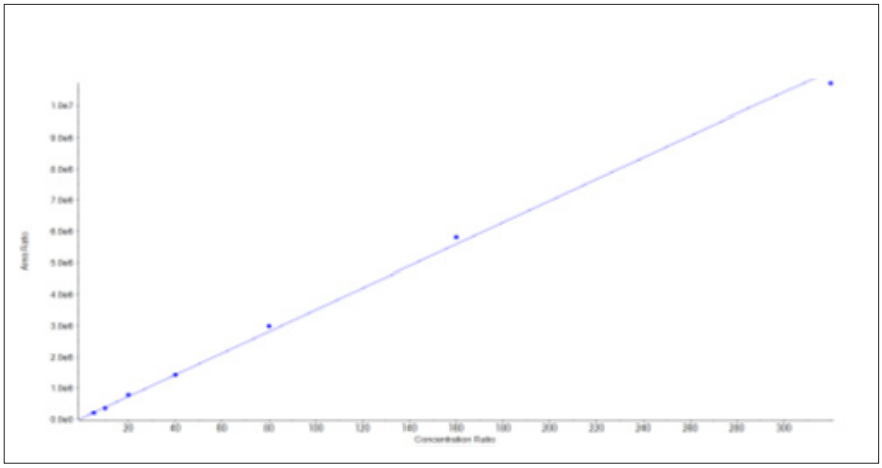

Figure 5: UHPLC-QTOF Calibration Curve for Sildenafil

UHPLC-QTOF Results for the Working Solution

Under the optimized chromatographic conditions, acceptable chromatographic separation of seven different sildenafil samples was achieved. It can be noted that the baseline separation can be reached for all 7 samples (m/z 475.212) as shown in (Figure. 6).

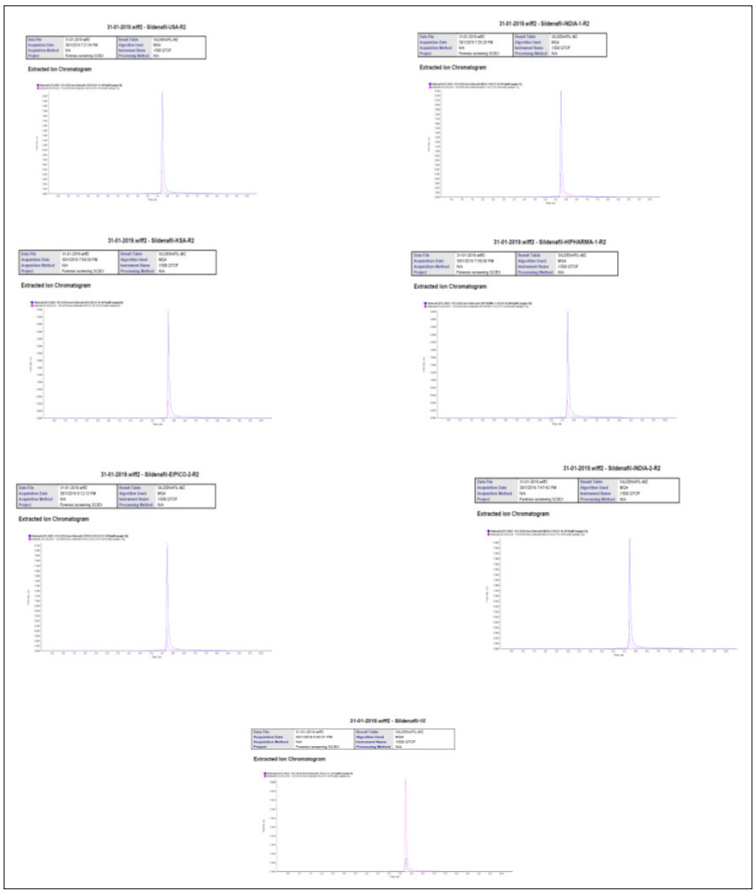

Figure 6: UHPLC-QTOF Chromatograms of Sildenafil Sample.

Table 6: MRM Transition Parameters of UHPLC-QTOF for the Determination of the Sildenafil Samples

		Sildenafil	T1(KSA)	T2(USA)	T3(IND 1)	T4(IND 2)	T5(HI)	T6(EPC)
Retention Time		5.75	5.74	5.74	5.74	5.73	5.72	5.73
m/z	Precursor Ion Experimental	475.212	475.212	475.212	475.211	475.211	475.212	475.212
	Precursor Ion Theoretical	475.212	475.212	475.212	475.212	475.212	475.212	475.212
	Product Ions	283.12 100.10	283.12 100.10	283.12 100.10	283.12 100.10	283.12 100.10	283.12 100.10	283.12 100.10

Discussion

There are many factors that require consideration for the selection of the method for analysis whether that is for a pharmaceutical product, a chemical class of product, many products of different chemical classes, or for inclusion of their transformation products (1, 5, 6)). These factors include, boiling point or polarity; solubility in desired solvent or mobile phases; stability of chemical product in injector ports, on-column, or in mass spectrometer ion sources; selectivity of columns and chromatographic behavior; interferences in detection; molecular structure or other chemical properties important for both ionization and fragmentation; method detection limit or regulatory requirements; and confirmation ability over linear dynamic range [7,8,9].

Selecting an analytical method for pharmaceutical authentication requires consideration of chemical properties, sample preparation complexity, detection sensitivity, and the nature of possible interferences. In this study, five techniques were compared for their ability to detect sildenafil across genuine, generic, and potentially falsified samples.

UV-Vis spectrophotometry proved to be a simple, cost-effective method for routine screening, with results following Beer–Lambert's law and showing consistent λmax values (~290 nm) across solvents. However, absorbance variations indicated differing sildenafil concentrations among samples, suggesting that while UV-Vis is effective for detection, it is less reliable for precise quantification in counterfeit analysis without further confirmation.

GC-MS/MS offered high specificity through multiple reaction monitoring (MRM) but was limited by the volatility and thermal stability requirements of the analyte. Although retention time reproducibility was strong, its applicability to a broad range of pharmaceuticals remains constrained.

HPLC-PDA provided flexibility and robust qualitative identification, with retention times between 5.03–5.10 minutes and the advantage of spectral confirmation. This method is suitable for quality control laboratories needing a balance of speed and reliability.

LC-MS/MS demonstrated the highest reliability and sensitivity for both detection and quantification. The ability to identify sildenafil and its structural analogues, combined with short retention times (~0.58 min) and high selectivity, makes LC-MS/MS the preferred option for forensic and regulatory applications.

UPLC-QTOF combined high-resolution mass measurement with accurate fragmentation fingerprinting, enabling confident compound identification. This method is particularly valuable for complex cases requiring unequivocal confirmation of authenticity and molecular profiling.
Overall, LC-MS/MS and UPLC-QTOF were the most effective methods for counterfeit detection, with UV-Vis suitable for rapid screening, HPLC-PDA for routine QC, and GC-MS/MS for selected applications.

The Main Results of this Study are Abstracted in the Following Table:

Parameter	UV-VIS	GC-MS/MS	LC-DAD	LC-MS/MS	LC-QTOF
Retention Time (min)	NA	13.0	5.3	0.58	5.72
Linearity Range	10-100 µg/ml	2.5-40 µg/ml	2.5-40 µg/ml	2.5-40 µg/ml	5.0-360 ng/ml
Slope	0.01188	0.002848	0.0504	1.15781	4.76426×10^4
Intercept	0.02229	0.002848	o.1205	0.48592	3.4699×10^4
Correlation Coefficient	0.99451	0.99751	0.99999	0.99787	0.99777

Conclusion

This comparative evaluation demonstrates that no single analytical technique universally addresses all counterfeit detection needs. LC-MS/MS and UPLC-QTOF offer the highest sensitivity, specificity, and confirmatory capabilities for sildenafil authentication, making them indispensable in forensic and regulatory contexts. UV-Vis remains valuable for preliminary screening due to its simplicity and low cost, while HPLC-PDA provides a reliable balance between speed and accuracy for routine use. GC-MS/MS, though effective for certain compounds, is less suited for non-volatile pharmaceuticals like sildenafil.

Integrating multiple analytical methods, as demonstrated in this study, offers a comprehensive approach to combating counterfeit pharmaceuticals. Such strategies enhance patient safety, protect industry integrity, and strengthen the ability of enforcement agencies to identify and remove falsified products from the market.

Declaration

1. Ethics approval and consent to participate: Not applicable
2. Consent for publication: All authors have read and agreed to the published version of the manuscript.
3. Availability of data and material: Data are contained within the article
4. Competing interests: The authors declare no conflict of interest
5. Funding: Not applicable

References

1. Mesheal AL-Abdulrahman, Yasir Abdullah bin Khaliffah, Muteap bahni alhaily, Meshael Oudah Dawi AlOtaibi, Abdul-Wali Ajlouni, et al. (2023) Detection Methods of Counterfeit Drugs: A Systemic Review. Journal of Pharmaceutical Research & Reports 4: 1-9.
2. Tesfaye W, Abrha S, Sinnollareddy M (2020) How Do We Combat Bogus Medicines in the Age of the COVID-19 Pandemic? The American Journal of Tropical Medicine and Hygiene 4: 1360-1363.
3. Bracci A, Nadini M, Aliapoulios M, McCoy D, Gray I, et al. (2021) Dark Web Marketplaces and COVID-19: before the vaccine. EPJ data science 1: 1-6.
4. Schneider M, Ho Tu Nam N (2020) Africa and counterfeit pharmaceuticals in the times of COVID-19. Journal of Intellectual Property Law & Practice 6: 417-418.
5. Gupta K, Singhal A (2012) Pandey Counterfeit (Fake) Drugs & New Technologies to Identify it in India. Int J Pharm Sci Res 11: 4057-4064.
6. Farmer KC (2020) Stress and strain on the U.S. drug supply: The intersection of shortages, globalization, counterfeit products, and throw in a global COVID-19 pandemic. J Am Pharm Assoc 61: 85-86.
7. Lakavage A (2020) Covid-19 has exposed cracks in the global medicines supply chain, we need to fix them, STAT 1-6.
8. UNODC (2020) Falsified Medicines in the Wake of COVID-19: An Emerging Threat for Security and Public Health 1-31.
9. Paola Bottoni, Sergio Caroli (2019) Fake pharmaceuticals: A review of current analytical approaches 149: 104053.