



LC-MS/MS detected lower values of dexamethasone and prednisolone in powdered herbal medicines sold in Tanzania

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Abstract

As a consequence of perceived quality and safety of herbal medicines in varied communities, adulteration of such medicines with synthetic drugs has become a growing global concern. This study assessed the adulteration of herbal medicines with dexamethasone and prednisolone using LC-MS/MS. The optimized method was used to confirm presence of conventional drugs (dexamethasone and/or prednisolone) in the fourteen samples suspected to be adulterated after screening with TLC. This method was validated by determining coefficient of determination, limit of detection and limit of quantitation. Coefficient of determination for dexamethasone and prednisolone were 0.95 and 0.97 respectively. The limit of detection for dexamethasone and prednisolone were 13.79 ppm and 10.81 ppm respectively. The limit of quantitation was 41 ppm and 32 ppm for dexamethasone and prednisolone respectively. The results indicated that all 14 samples were adulterated with either all or one of the two suspected adulterants. It was observed that, while both prednisolone and dexamethasone were detected in nine herbal medicines samples, one (1) sample was adulterated with dexamethasone alone and four (4) samples were adulterated with prednisolone alone. However, the dexamethasone and prednisolone adulterants were only detected but could not be quantified because they were below quantification limit. The optimized method meets the International Council for Harmonization (ICH) standards therefore can be used to confirm the presence of dexamethasone and prednisolone in herbal medicines.

Key words: *Adulteration; dexamethasone; herbal medicines; LC-MS/MS; prednisolone*

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Introduction

Herbal medicines have gained a lot of attention due to the perception that they are less expensive, safer and healthier than synthetic drugs (Karimi *et al.*, 2015; Zhou *et al.*, 2016; Ariffin *et al.*, 2021). Recently, the demand of herbal medicines has increased (Ekor, 2014), this has brought the rapid growth of herbal medicines market which in other side has influenced the adulteration cases. There are unlawful practices from traditional practitioners where they tend to mix herbal medicines with synthetic drugs (Lawati *et al.*, 2017; Mpanyakavili *et al.*, 2022; Mwankuna *et al.*, 2022; Walusansa *et al.*, 2022). Among of synthetic drugs found in various countries to be adulterated in herbal medicines are corticosteroid drugs (Limmatvapirat *et al.*, 2012; Ofori-Kwakye *et al.*, 2014; Park *et al.*, 2016; Ahmed and Hassan, 2020; Permatasari *et al.*, 2021; Pratiwi *et al.*, 2022). The prolonged use of corticosteroids unknowingly expose risk to the consumers health (Permatasari *et al.*, 2021). Adverse effects such as skin atrophy, cutaneous reactivity, hypertension, diabetes mellitus, osteoporosis and Cushing's syndrome might arise with long term use of corticosteroids (Limmatvapirat *et al.*, 2012; Park *et al.*, 2016; Zhou *et al.*, 2016; Ariffin *et al.*, 2021). In Tanzania, where the herbal medicines market is thriving, there is an urgent need for rapid detection methods to detect these adulterants.

Synthetic corticosteroid medications such as betamethasone, cortisone, dexamethasone, prednisone and prednisolone are usually used as anti-inflammatory drugs to minimize inflammation, treat allergies, aid in organ transplantation, helps in treating respiratory infections, arthritis, joint pain and body balance (Ekor, 2014). Of these corticosteroids, dexamethasone and prednisolone were commonly used as over the counter drugs during COVID-19 pandemic (Ekor, 2014; Ahmed and Hassan, 2020; Johnson and Vinetz, 2020; Ortiz *et al.*, 2021). This may have persuaded dishonest traditional practitioners to add dexamethasone and or prednisolone in herbal medicines. However, adulteration of herbal medicines with dexamethasone and or prednisolone in Tanzania remains poorly documented.

Variety of analytical techniques has been applied to detect adulterants in herbal medicines including thin layer chromatography (TLC), Gas chromatography-Mass spectrometry (GC-MS), Ultra high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) and Liquid chromatography connected to Mass spectrometry/Mass Spectrometry (LC-MS/MS). Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has emerged as indispensable analytical technique to perform routine chemical analysis, in forensic science also in confirmation of adulterants in herbal medicines, food supplements and cosmetics (Limmatvapirat *et al.*, 2012; Ofori-Kwakye *et al.*, 2014; Park *et al.*, 2016; Giaccone *et al.*, 2017). LC-MS/MS is a potent analytical tool for performing both qualitative and quantitative analysis. Separation, detection and quantitation are all made possible by the dual selectivity of the instrument (Vaclavik *et al.*, 2014; Mpanyakavili *et al.*, 2022). The screening of 423 herbal medicines collected in different regions in Tanzania showed a likelihood of adulteration of some herbal medicines with dexamethasone and or prednisolone (Kibiki *et al.*, 2023). This calls for performing an analysis to confirm presence of such conventional drugs in herbal medicines sold in Tanzanian market. Due to herbal medicine market's growth in Tanzania, there is an urgent need for more sensitive analytical tools such LC-MS/MS to detect and confirm these corticosteroids adulterants in herbal medicines.

Materials and methods

Chemicals and Reagents

Dexamethasone (0.5 mg- DEXAMED) and prednisolone (5 mg-PREDILONE) tablets were purchased from authorized pharmacies, HPLC grade solvents including methanol (Finar, India, 99.8%), acetonitrile (Finar, India, 95%) and water (Carlo Erba, France) were used.

Preparation of Stock and Standard Solutions

Stock solution of 1000 ppm of each dexamethasone and prednisolone were prepared in methanol. Dilutions were done using the same solvent to obtain standards solutions of 2 ppm, 20 ppm and 200 ppm. Finally, the

300 μ L of each standard solution was diluted with 700 μ L of water to increase the aqueous content in sample which is suitable for HPLC analysis.

LC and MS/MS Instrumentation Conditions

Dexamethasone and prednisolone were separated using Agilent 1100 High performance liquid chromatography equipped with degasser G139A, binary pump G1312A, autosampler G1313A, Column oven G1316A, diode array detector G1315B and the analytes were separated using analytical column: Kinetex C_{18} 2.6 μm 100 \AA (75 X 2.1 mm). Column temperature was maintained at 40 $^{\circ}\text{C}$, Flow rate was set to 0.3 ml/min, injection volume of sample was 5 μL . Mobile phase used composed of mobile phase A with 0.1% formic acid in water and mobile phase B with 0.1% formic acid in acetonitrile. Gradient elution started with 30% of mobile phase B, this composition was maintained for 3.5 min, after this it was linearly increased to 70% B over 0.5 min, then increased to 95% then 95%B was held constant for 1.1 min to equilibrate HPLC system. Then the column was set back to its initial composition over 6.4 min. The total run time was 12 minutes.

MS used was a triple quadrupole mass spectrometry (Waters Micro-mass UK Limited) equipped with an ESI interface operated in positive mode. Mass lynx 4.1 software was used to operate the MS system. Nitrogen gas was used as nebulization gas and Argon gas was used as collision gas. The ion source parameters used were: desolvation temperature 400 $^{\circ}\text{C}$; Source temperature 120 $^{\circ}\text{C}$; Radio frequency lens 0.3 kV; exit potential 2 v, capillary voltage 3 kV; desolvation gas flow and cone gas flow were 650 L/h and 100 L/h respectively. Collision voltage, cone voltage used, precursor ion, qualifier and quantifier are shown in Table 2.

Checking for Standards Purity

The purified standards were subjected into HPLC for analysis. Peak area obtained in chromatogram was used to show percentage purity and retention time was used for confirmation.

Confirmation of Standards

A stock solution of 1000 ppm for each dexamethasone and prednisolone was prepared. From stock solution 200 ppm was prepared in mobile phase then directly infused in MS machine. The full MS scan was performed to see the molecular ion for each targeted analyte.

Establishment of Calibration Curve, Limit of Detection (LOD), Limit of Quantitation (LOQ) and Precision Calibration Curve

Linearity was determined by plotting the peak area of the analytes versus concentrations of the analytes. To determine the adulterated herbal medicines, the calibration curve from concentrations of 2, 5, 20, 40, 60, 80, 100 and 120 ppm of dexamethasone and prednisolone were prepared. The calibration curves were best fitted using a least-square linear regression model $y = mx + c$ in which y is the peak area of the analyte, m is the

slope of the calibration curve; c is y-axis intercept of the calibration curve and x is concentration of analyte.

Limit of Detection

The limit of detection (LOD) of the method was determined from the calibration curve using Equation 1

$$LOD = 3.3 \times \text{Standard deviation} \quad \text{Equation 1}$$

Limit of Quantitation

The limit of quantification (LOQ) of the method was determined from the calibration curve using Equation 2

$$LOQ = 10 \times \text{Standard deviation} \quad \text{Equation 2}$$

Precision

The precision was determined in terms of intra-day repeatability. The precision was expressed as relative standard deviation (RSD) which was calculated using Equation 3

$$RSD = \frac{\text{Standard deviation}}{\text{Mean}} \times 100\% \quad \text{Equation 3}$$

Sample Selection and Pre-treatment

The screening of herbal medicines conducted by (Kibiki *et al.*, 2023) found that fourteen (14) samples of powdered herbal medicines were probably adulterated with dexamethasone and or prednisolone. The LC-MS/MS was used to confirm the adulteration of the 14 herbal medicine samples with dexamethasone and or prednisolone.

Sample Preparation and Analysis

Approximately 2 g of herbal medicine powder was dissolved in 10 ml of methanol. Samples were sonicated for 30 minutes and centrifuged at 4000 rpm for 15 minutes. The supernatant was taken into empty vial then solvent was left to evaporate under room temperature. The dried sample was mixed with 1 ml of methanol then filtered using microporous filter 0.22 μm , 300 μL of the sample was diluted with 700 μL of HPLC grade water and injected into LC-MS/MS. Each sample was analyzed in duplicate.

Data Analysis

Data was analyzed using descriptive statistics and regression analysis. Descriptive statistics was used in finding mean and standard deviation which were useful in calculating LOD, LOQ and precision. Regression analysis was conducted to explore the relationship between peak area of the analyte and concentration of the analyte where coefficient of determination and linear equation was obtained.

Results

Checking Purity

Percentage purity obtained for dexamethasone was 98% and for prednisolone was 88%. The tallest peak was observed at retention time 1.026 min for dexamethasone as shown in Figure 1 and that of prednisolone were observed at 0.828 min as shown in Figure 2.

Figure 1

A chromatogram of dexamethasone standard (Absorbance versus retention time).

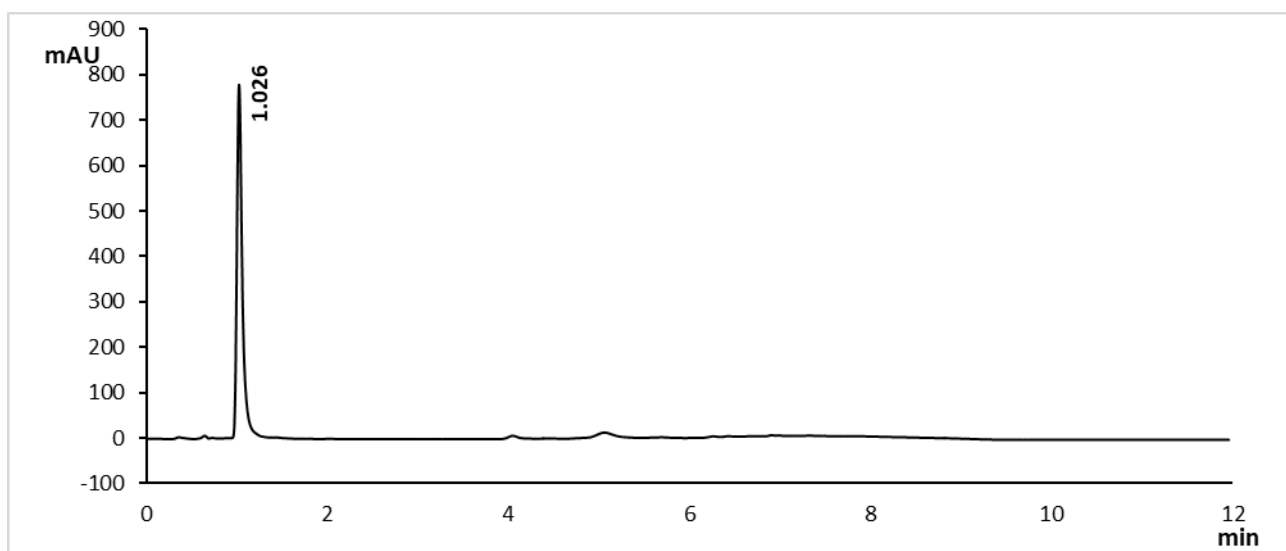
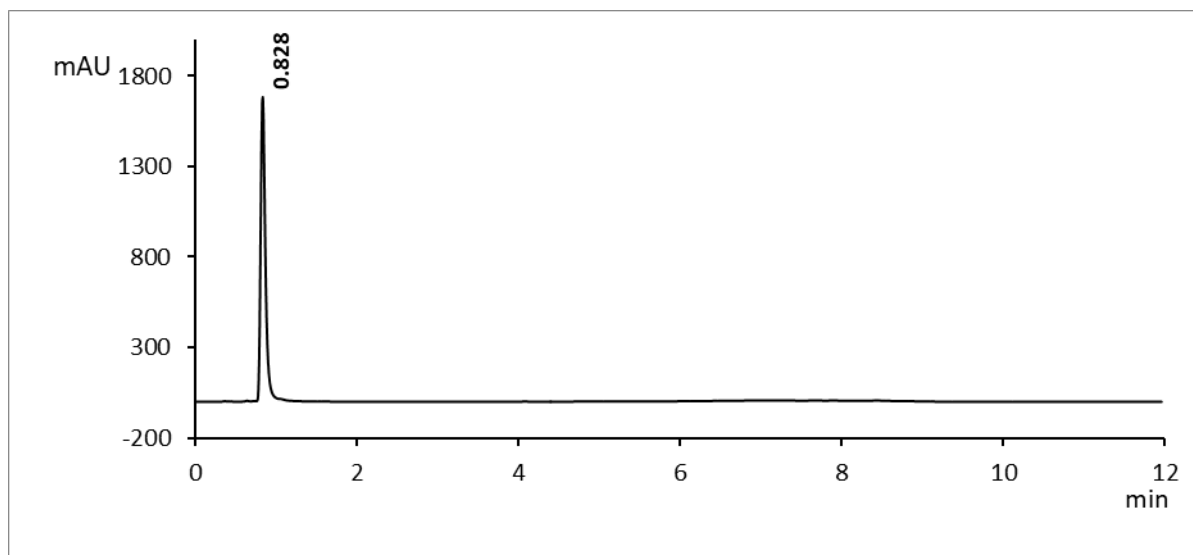


Figure 2

A chromatogram of prednisolone standard (Absorbance versus Retention time)



Standards Confirmation

The molecular ions of standards obtained at the optimized parameters were similar to dexamethasone and prednisolone molecular ions. The peaks (393.41, 394.43 and 395.44) m/z in dexamethasone spectrum as observed in

Figure 3 and (361.33, 362.29 and 363.30) m/z in prednisolone spectrum as observed in *Figure 4*.

Figure 3

An MS spectrum of prednisolone standard produced from mass lynx 4.1

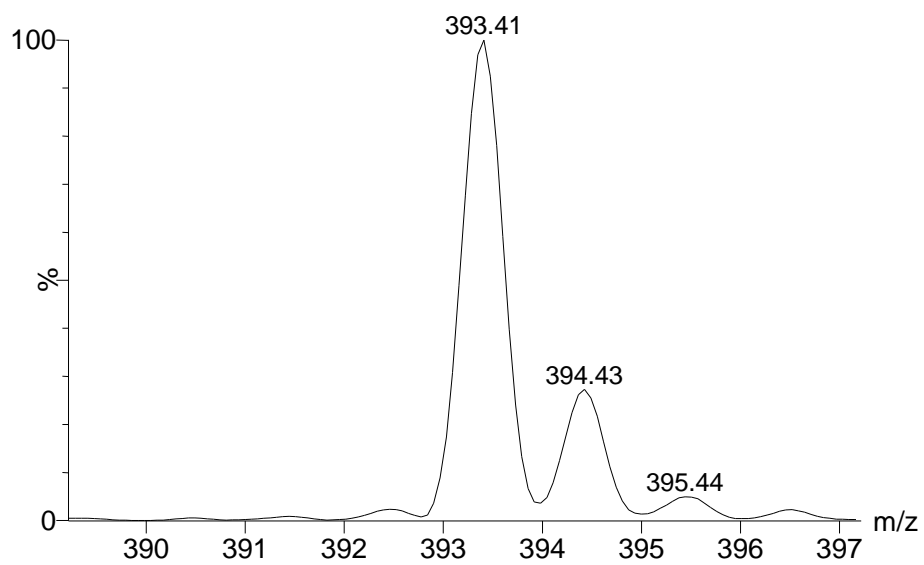
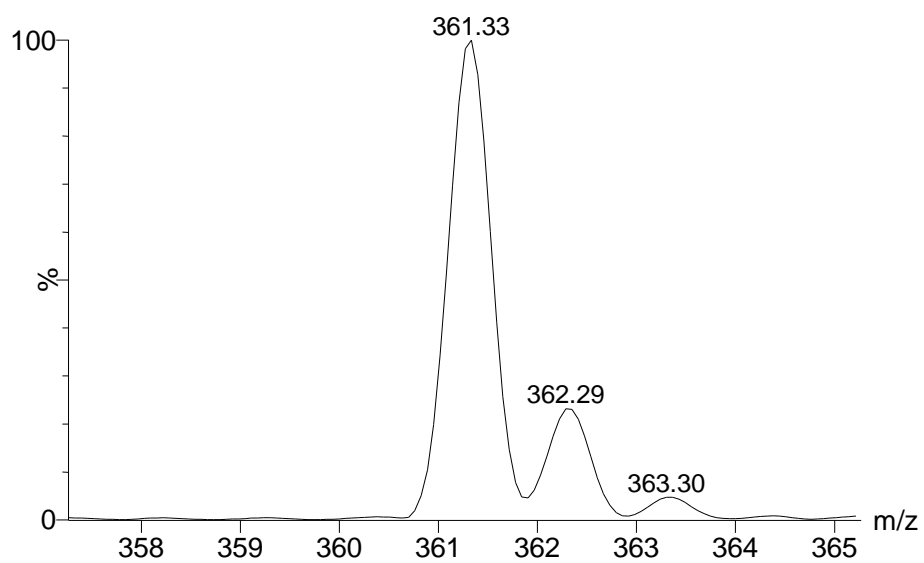


Figure 4

An MS spectrum of dexamethasone standard produced from mass lynx 4.1



quantitatively showed correct results. The prepared standard solutions were analyzed and calibration curves were drawn. The slope equation and R- square values of the calibration curves obtained are shown in the

Linearity, LOD and LOQ

The calibration curve was used to determine the linear range and the linear range was used to obtain the concentration range at which the method

Table 1. The limit of detection for dexamethasone and prednisolone were 13.79 ppm and 10.81 ppm respectively.

Table 1

Linear equation, Precision, R-square, LOD and LOQ

Corticosteroid name	R ²	Equation of line	LOD (ppm)	LOQ (ppm)	Precision
Dexamethasone	0.9491	Y=25.528X +131	13.79	41.78	5.43
Prednisolone	0.9688	Y=156.18X +1337	10.81	32.75	7.55

Optimized MS/MS Parameters

Precursor ions were generated through MS scans of dexamethasone and prednisolone in positive ion mode. Each precursor ion was fragmented into three fragment ions and two fragment's ions with highest abundance were selected. The Multiple Reaction Monitoring (MRM) transition for the most abundant product

ion (base peak) was selected as quantifier, and the second highest abundance was selected as qualifier for the targeted compounds. The cone voltage used to fragment precursor ions, and collision energy suitable for each fragment is listed in

Table 2.

Table 2

MRM transition parameters of MS/MS for detecting dexamethasone and prednisolone

Compound	Precursor ion (m/z)	Cone voltage (kV)	Product ion (m/z)	Collision energy (kV)
Dexamethasone	393.22	17	337	13

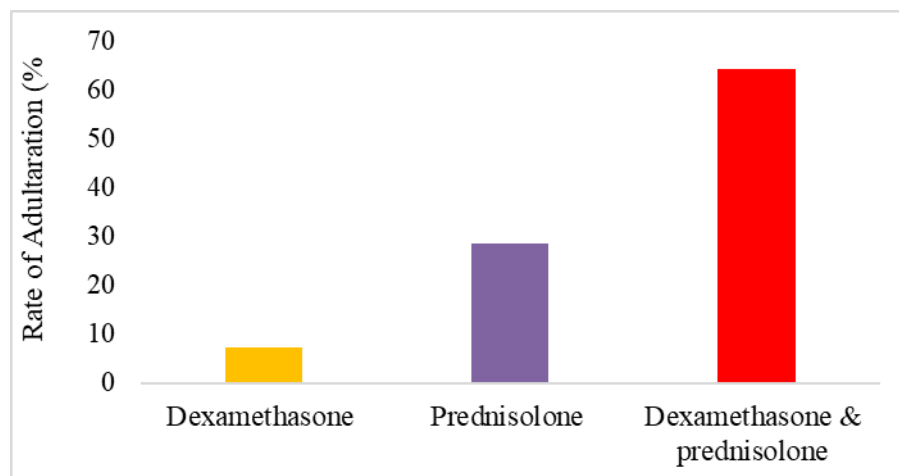
			355	12
			373	10
Prednisolone	361.33	16	146.9	22
			325.1	10
			343.1	9

LC-MS/MS Analysis of Herbal Medicines

The optimized method was applied in each herbal sample for detection and quantitation of dexamethasone and prednisolone. Among 14 screened herbal samples, dexamethasone was detected in one herbal sample only (7%), prednisolone was detected in four herbal samples (24%) while in nine herbal samples (79%) both dexamethasone and prednisolone adulterant were detected (Figure 5).

Figure 5

Corticosteroid adulterant detected in herbal samples



Discussion

According to International Community Harmonization (ICH) guideline the degree of purity necessary for the reference material depend on the intended use (ICH, 2022). Therefore, these standards had minimum impurities hence were acceptable for analytical purposes. The peaks (393.41, 394.43 and 395.57) m/z in MS spectrum for dexamethasone and (361.33, 362.29 and 363.43) m/z in MS spectrum for prednisolone are due to existence of different isotopes of carbon regarding to their relative abundances. Carbon-12 and carbon-13 are the stable isotopes while carbon-14 is unstable isotope which is a nuclide.

The LOD and LOQ obtained in this study are similar to detected limit of 13.13 ppm reported by (Pratiwi *et al.*, 2023) who used polymethylmethacrylate strips to detect dexamethasone. Compared with other researchers (Park *et al.*, 2016; Jin *et al.*, 2017) who used LC-MS/MS for detection of dexamethasone and prednisolone in herbal medicines, the LOD and LOQ values obtained in this study were high. Using LC-MS/MS the reported LOD value for dexamethasone and prednisolone ranged from

0.00188ppm to 2 ppm (Park *et al.*, 2016; Jin *et al.*, 2017). This could be due to the preparations steps of standards used which were purified directly from the tablets. Also additional steps in sample preparation and analysis, such as removing unwanted matrix component, derivatization of the analyte, further dilution of the sample and concentrating the sample prior to analysis were reported to affect LOD and LOQ values (Sheehan and Yost, 2015; Wells *et al.*, n.d.).

The optimized method was applied in each herbal sample for detection and quantitation of dexamethasone and prednisolone. The adulterants were only detected but were not quantifiable, the concentration was below the limit of quantification. Other studies has reported that adulteration of herbal medicines with synthetic drugs can intentionally be done to increase the efficacy of those medicines (Park *et al.*, 2016; Permatasari *et al.*, 2021). This unlawfully practice can also be done by wholesalers, formulators, retailers and healers (Zhang *et al.*, 2012). The adulteration of herbal medicines with corticosteroids drugs is linked with low prices of these drugs (Anwar *et al.*, 2023). Adulteration of herbal medicines with corticosteroids can lead to herbal-drug interaction which can have either

synergistic or antagonistic effects (Kuswandi *et al.*, 2021; Kintoko *et al.*, 2023). Some of the adverse effect caused by using herbal medicines adulterated with corticosteroids drugs include weight gain, increased blood sugar level and Cushing syndrome (Haneef *et al.*, 2013). Worldwide, there are many studies detected dexamethasone and prednisolone in herbal medicines (Friedrich *et al.*, 2009; Limmatvapirat *et al.*, 2012; Garza-ocañas *et al.*, 2013; Park *et al.*, 2016; Zhou *et al.*, 2016; Asra *et al.*, 2018; Li *et al.*, 2018; Permatasari *et al.*, 2021; Pratiwi *et al.*, 2022). However, this is the first study to report the existence of adulteration of dexamethasone and prednisolone in herbal medicine sold in the Tanzania market. According to WHO herbal medicines which include undeclared conventional drugs are illegal, also those medicines containing both herbal medicines and conventional drugs should not be considered as herbal medicines (WHO, 2003; Zhang *et al.*, 2012).

Generally, the method optimized is suitable for determining dexamethasone and prednisolone drugs in herbal medicines. The combination of LC with tandem mass spectrometry offers a reliable method for identifying trace levels of dexamethasone and prednisolone in the presence of interfering compounds. The use of qualifiers in MS enabled the distinction of dexamethasone and prednisolone from similar compounds present in herbal medicines hence reducing the likelihood of false positives. Therefore, by comparing the retention times, mass spectrum and relative abundances of selected fragment ions with reference standards, the method can accurately detect and quantify the presence of dexamethasone and prednisolone in herbal medicines.

Conclusion

The LC-MS/MS method optimized was successfully applied in detecting adulterants in herbal medicines. The fact that the corticosteroid adulterants were not declared legally as one of the ingredients in herbal medicine samples, the fourteen samples were confirmed adulterated. This is an alarm to drug authorities to make effort to check herbal medicines quality before they reach to the consumers.

Recommendation

The LC-MS/MS method optimized might be applied as part of regulatory practices to verify the presence of dexamethasone and prednisolone in herbal medicines. Also, education should be provided to herbal practitioners on the risk of adulterating herbal drugs with synthetic drugs, herbal users should be alerted on using unregistered herbal drugs. Also, continuous development of screening protocols for herbal medicines is essential, since the adulteration may pose a threat and cause adverse effects.

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Declaration of interest

None.

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