
Rapid Detection of Corticosteroids in Herbal Medicines Sold in Tanzania

Kibiki, A.E.*, A.L. Mpanyakavili, C.J. Mwankuna, E.E. Mariki and F.P. Mabiki

Department of Chemistry and Physics, College of Natural and Applied Sciences,
Sokoine University of Agriculture, Morogoro, Tanzania

*Corresponding author email: anna.kibiki@student.suanet.ac.tz. Phone: +255656266590

Abstract

Adulteration of herbal medicines with corticosteroid drugs poses risk to health of consumers. The broad-spectrum drugs including dexamethasone and prednisolone which were mostly used during Corona virus disease (COVID-19) era have been reported as common adulterants in herbal medicines. The increase of herbal medicine market in Tanzania calls for optimization of simple and rapid method for detection of corticosteroids adulterants in these drugs. Four hundred and twenty-three archived herbal samples were analysed using optimized Thin Layer Chromatographic (TLC) method. Retention factor value and visualization reagent were used for confirmation of adulterants in herbal medicines. Results report that 28.13% of all analysed herbal samples were adulterated while 71.87% of all samples no corticosteroid adulterant found. Among herbal samples found with corticosteroids, 87.39% had one adulterant while 12.61% contained at least two adulterants. The optimal concentration for visualization reagent is using equal volume of 3 mol/dm³ sodium hydroxide and 0.625 mg/mL of tetrazolium blue chloride while the limit of detection was 0.26 mg/ml for prednisolone and 2.62 mg/ml for dexamethasone. A rapid method for the detection of dexamethasone and prednisolone in herbal medicines was developed and optimized to determine the presence of these corticosteroids in herbal samples. The method is recommended for use before confirmatory test such as liquid chromatography connected to tandem mass spectrometry (LC-MS/MS).

Keywords: Dexamethasone; Prednisolone; Herbal Medicines; Adulterants

Introduction

Herbal medicines have played a fundamental role in animal and human health care for countless years. Nowadays, the demand for herbal medicines as an alternative to conventional drugs is on increase partly because herbal medicines are considered by most communities to be safe compared to the conventional drugs (Karimi *et al.*, 2015; Permatasari *et al.*, 2021). The herbal medicines business in Tanzania is lucrative such that some herbal practitioners adulterate their medicines with conventional drugs to enhance stated effect and benefit from business (Mpelangwa *et al.*, 2022; Mwankuna *et al.*, 2022; Permatasari *et al.*, 2021; Pratiwi *et al.*, 2022). Adulteration is done using cheaply available conventional drugs over the counter drugs or broad spectrum

drugs have been conducted in Tanzania, to detect herbal medicines adulterated with pain killers, anti-malaria, antibiotics and drugs used to enhance male sexual desire and penile enlargement (Mpanyakavili *et al.*, 2022; Mwankuna *et al.*, 2022, 2023; Otieno *et al.*, 2014). However, adulteration of herbal medicines with conventional corticosteroids remains poorly assessed.

Corticosteroids are among the anti-inflammatory commonly used for treatment of respiratory infections (such as asthma), allergies, inflammation, low back pain, and hypertensive disorders during pregnancy (Liwa *et al.*, 2017; MoHCDEC, 2018; Walusansa *et al.*, 2022). Dexamethasone and prednisolone, being the broad-spectrum corticosteroid drugs have been reported to be adulterated in herbal medicines (Anwar *et al.*, 2023; Asra *et al.*, 2018). Studies

carried out in Thailand, Malaysia and China revealed dexamethasone and prednisolone to be adulterated in herbal medicine especially those targeting arthritis and muscle pain. Adulteration of herbal medicines with corticosteroid drugs are likely to cause human health problems such as liver damage, kidney failure, peptic ulcers, Cushing's syndrome and sometimes death (Ariffin *et al.*, 2021; Limmatvapirat *et al.*, 2012; Park *et al.*, 2016). In Tanzania, where the herbal medicines market is thriving, there is an urgent need for rapid detection methods.

Dexamethasone and prednisolone were acknowledged as a substantial advancement in the fight against COVID-19 and dramatically decreased the death rate among instances of COVID-19 that were considered to be severe (Ahmed & Hassan, 2020; Johnson & Vinetz, 2020; Noreen *et al.*, 2021). The use of these drugs during COVID-19 era exposed them to the extent that they become common/ familiar drug. This might have convinced untrustful traditional practitioners to mix with their herbal medicines. This made the choice of two corticosteroids drugs.

Dexamethasone ($C_{22}H_{29}FO_5$) and prednisolone ($C_{21}H_{28}O_5$) (Fig. 1) are less conjugated compounds which can be observed under ultra violet (UV) light at short wavelength of 254 nm and appear as dark spot under green background due to quenching (Limmatvapirat *et al.*, 2012; Pyka, 2014). When sprayed with tetrazolium blue solution the compounds give a violet colour as a confirmatory test for corticosteroid compounds (Limmatvapirat *et al.*, 2012). The solubility of prednisolone in water is 0.2 mg/mL while that of dexamethasone is less than 0.1 mg/mL at 25 °C (Klauson *et al.*,

2013). They are soluble in organic solvents. This study used UV light (physical method) and colour reaction (chemical method) to detect corticosteroids in herbal medicines that are sold in markets in nine regions of Tanzania.

Different analytical techniques including TLC, high performance liquid chromatography (HPLC), liquid chromatography connected to tandem mass spectrometry (LC-MS/MS) and gas chromatography-mass spectrometry (GC-MS) have been reported to be used in screening of corticosteroids in herbal medicines (Haneef *et al.*, 2013; Mpanyakavili *et al.*, 2022; Vaclavik *et al.*, 2014). Thin layer chromatography has been mentioned to be simple and a cheap compared to other techniques (Li *et al.*, 2018; Mwankuna *et al.*, 2023; Pyka, 2014). It can be applied in the field as the sample can be analysed in the absence of electric power using colour reaction (Satcher *et al.*, 2012). Analysis using TLC does not necessitate high purity and concentrated samples (Pyka, 2014). Also it can be used when HPLC and GC are not appropriate, such as when the studied compound has no UV activity or the compound is not volatile (Pyka, 2014). In contrast HPLC, LC-MS/MS and GC/MS techniques are expensive, requires an expert to operate and cannot be carried in the field (Friedrich *et al.*, 2009; Mwankuna *et al.*, 2023). This makes analysis of many herbal samples directly using sophisticated methods such as LC-MS/MS and GC-MS time consuming and expensive (Li *et al.*, 2018). Thus, rapid detection methods for screening herbal medicines which are simple and cheap are required. The combination of thin layer chromatography with other method such as TLC-densitometry (Permatasari *et al.*, 2021), TLC- visible spectrophotometer (Pratiwi

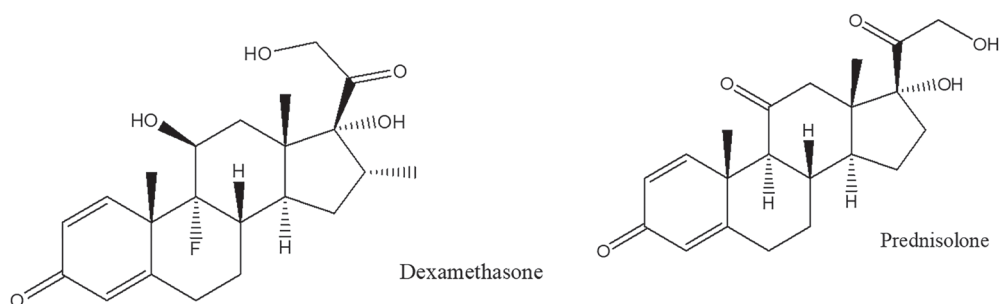


Figure 1: Chemical structures of dexamethasone (392 g/mol) and prednisolone (360 g/mol)

et al., 2022) and TLC combined with spot concentrated Raman scattering (Li *et al.*, 2018) in detecting adulterants in herbal medicines have also been reported. This is among the effort to get analytical methods which are simple and inexpensive. Therefore, the purpose of this study was to optimize the available TLC method for rapid detection of corticosteroid adulterants in herbal medicines.

Materials and Methods

Materials, reagents and chemicals

Dexamethasone (0.5 mg-DEXAMED) and prednisolone (5 mg-PREDILONE) tablets were purchase from authorized pharmacies, HPLC grade solvents including methanol (Finar, India, 99.8%), acetonitrile (Finar, India, 95%) and water (Carlo Erba, France) were used. Also, ethanol (analytical grade), dichloromethane (Loba chemie, India), ethyl acetate (Loba chemie, India), cyclohexane (Rochelle, South Africa), formic acid (Finar, India), sodium hydroxide (97%), and TLC silica gel 60 F254, analytical balance (BYY 21, Germany) were used. Tetrazolium blue chloride (95%) was purchased from Loba chemie, India. The UV lamp was operated at 254 nm. The archived powdered herbal samples obtained from chemistry laboratory in department of chemistry and physics at Sokoine University of Agriculture were used.

Preparation of reagents

Preparation of standards

Standards of dexamethasone and prednisolone were extracted from tablets and confirmed by high performance liquid chromatography- tandem mass spectrometer (HPLC-MS/MS).

Extraction of standard from tablets

Seven solvents (cyclohexane, dichloromethane, ethanol, water, methanol and ethyl acetate) were tested for extracting standards. To individual test tubes containing 5 mL of a solvent one tablet of either dexamethasone or prednisolone was added, (n=3 for each solvent). The mixture was vortex mixed for one minute, allowed to settle and 3 mL of supernatant were transferred to a 5 mL

vial. Each of the extracts were spotted on TLC plate to evaluate the extraction process. The effectiveness of the solvent in extraction was evaluated by the presence or absence a spot on the TLC plate.

Concentrating extract and purification of standards

Two microliters of each supernatant of the standards which showed single spot were dried then dissolved with methanol to make a solution. TLC was used to check the qualitative purity of the standard, where 2 μ L from each prepared standard were spotted on TLC and eluted with ten percent of methanol in dichloromethane. Solvents which resulted into high yield of extract were used to extract standards.

Confirmation of extracted standards

The extracts of dexamethasone and prednisolone that showed spots on TLC plates were subjected to LC-MS/MS for confirmation.

Preparation of visualization reagent

The visualization reagent was prepared by mixing equal volumes of tetrazolium blue solution and sodium hydroxide solution. The tetrazolium blue solution was prepared by weighing masses of 0.5 g, 0.05 g, 0.025 g, 0.0125 g and 0.00625 g of tetrazolium blue chloride and dissolved in a mixture of 1:1 v/v of water and ethanol. 100 mL of 6 mol/dm³ and 3 mol/dm³ of sodium hydroxide aqueous solution were prepared.

The use of visualization reagent

Different concentrations of the solutions were tested to obtain the concentrations which give the suitable result and use small amount of tetrazolium blue chloride. The smallest concentration of tetrazolium blue solution which was able to show violet colour clearly in colour reaction was taken as optimal concentration.

Preparation of elution solvent

The elution solvent system was prepared according to Limmatvapirat *et al.*, (2012), where dichloromethane contained 10% methanol and 0.005% formic acid.

TLC methods of detection and visualization

The developed TLC plates were visualized under UV lamp at 254 nm as well as after spraying with a reagent containing tetrazolium blue and sodium hydroxide solutions.

Determination of limit of detection of standards

A mixture of standards (2.1 mg/mL prednisolone and 21 mg/mL dexamethasone) was serially diluted and after each dilution 2 μ L of the mixture was spotted on TLC plate and eluted. The plate was observed under UV at 254 nm and sprayed with spraying reagent. The lowest concentrations for which spots were visible under UV at 254 nm and showed violet colour after spraying were considered as limits of detection.

Sample extraction

Sample extraction was carried out as per (Park *et al.*, 2016) with slight modification. One gram of herbal sample was dissolved in 5 mL methanol. Samples were vortexed for 30 seconds then centrifuged at 4000 rpm for 15 minutes. The supernatant was stored in Eppendorf tube, ready for analysis.

Sample analysis TLC

Two microliters of each the extracted herbal samples were spotted on the TLC plate (20 x 6.7 cm) at a distance of 1 cm along with a mixture of standards at the beginning and the end of spots. The plates were developed using dichloromethane containing 10% methanol and 0.005% formic acid. The developing solvent was allowed to saturate for 30 minutes prior to development. After elution the TLC plate was allowed to dry for one minute and was then observed under UV lamp at 254 nm to locate the separated spots. Thereafter, the TLC plate was sprayed with visualization reagent and heated at 105 °C for the best colour development. The retention factor (R_f) values for each spot were calculated using Equation 1.

$$\text{Retention factor (R}_f\text{)} = \frac{\text{Distance moved by analyte}}{\text{Distance moved by mobile phase}}$$

Consideration of adulterants in herbal medicines

Similarity of retention factors of sample spots and standard spots as observed under UV at 254 nm as well as formation of violet colour after spraying and heating saved as identity of dexamethasone and prednisolone in herbal samples.

Results

Selected extraction solvent for extraction of standard

A single spot was observed during TLC analysis after extracting dexamethasone with methanol, ethanol, dichloromethane, acetonitrile, ethyl acetate and water. A single spot of prednisolone was achieved by using acetonitrile and ethanol while dichloromethane, ethyl acetate and methanol extracts showed two spots. The single spots consistently showed a violet colour after reaction with tetrazolium blue reagent and (R_f) mean value was 0.54 and 0.48 which are similar with the R_f value of the standard dexamethasone and prednisolone respectively. Cyclohexane could not dissolve any compound in the tablets, so it indicated no spot during TLC elution.

Concentrating extract and purification of standards

The highest yield of dexamethasone was achieved while extracting with methanol while for prednisolone higher yield were achieved by extracting with acetonitrile tablet contains dexamethasone (0.5 mg) which is active ingredients.

Confirmation of the extracted drugs

The spectrum obtained from LC-MS/MS (Figure 2 and Figure 3) were used to confirm the purified standards. The precursor ion with the most abundant signal obtained was 437 m/z for dexamethasone and 405 m/z for prednisolone and each molecular ion composed of formate adduct [M+HCOO]⁻. The molecular ion peak was used to confirm the extracted drugs and qualify those extracted drugs to be used as standards for dexamethasone and prednisolone.

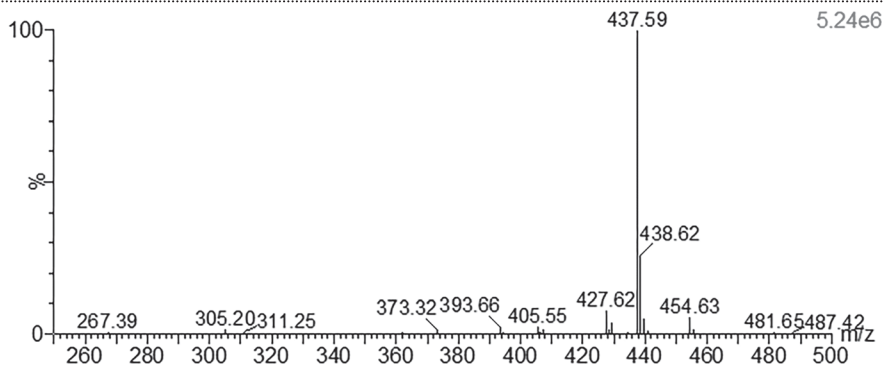


Figure 2: An MS spectrum for dexamethasone standard

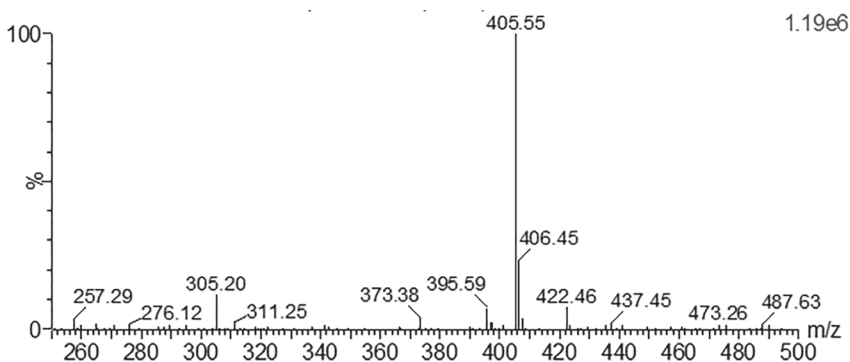


Figure 3: An MS spectrum for prednisolone standard

Optimized concentration for TLC visualization reagent

The optimal concentration for spraying reagent was found to be mixing equal volume of 3 mol/dm³ sodium hydroxide and 0.625 mg/mL tetrazolium solutions, respectively.

Application of the method

The results indicate that of all samples analysed (Appendix 1), 40% were adulterated with dexamethasone while 45% herbal samples were adulterated with prednisolone. 8% of herbal samples were adulterated with

Table 1: Concentrations tested to get the optimal concentration for TLC visualization reagent

Sodium hydroxide solution (mol/dm ³)	Tetrazolium blue solution (mg/mL)	Colour observed after colour reaction	TLC disintegration
6	10	Violet	Disintegrated
6	5	Violet	Disintegrated
6	0	No colour	Disintegrated
3	5	Violet	Not disintegrated
3	2.5	Violet	Not disintegrated
3	1.25	Violet	Not disintegrated
3	0.625	Violet	Not disintegrated
3	0.3125	Violet	Not disintegrated

Detection limit of standards

Detection limit of dexamethasone and prednisolone standards under UV at 254 nm were 0.263 mg/mL for prednisolone and 2.625 mg/mL for dexamethasone.

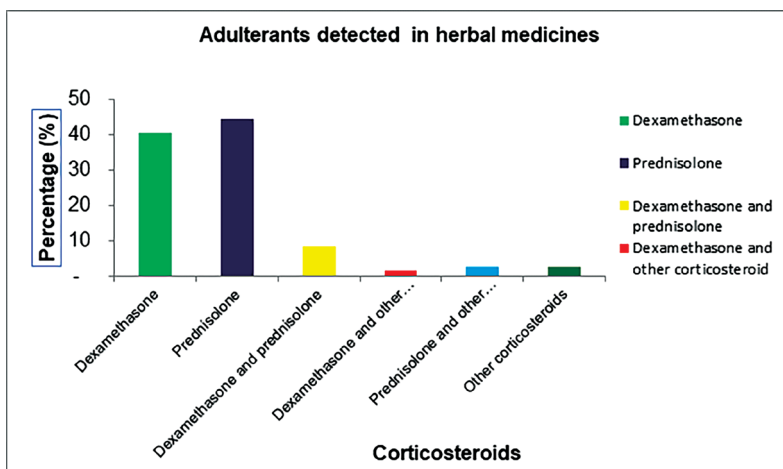
both dexamethasone and prednisolone; 2% adulterated with dexamethasone and other corticosteroid adulterants; 3% contained prednisolone and other corticosteroids, and 3% contained other corticosteroids which is neither

Table 2: Various concentrations for prednisolone and dexamethasone standards observed under UV (254 nm) and colour reaction.

S/N	Prednisolone (mg/mL)	Dexamethasone (mg/mL)	UV 254 (nm)	Colour reaction
1	2.100	21.000	Positive	Violet
2	1.050	10.500	Positive	Violet
3	0.525	5.250	Positive	Violet
4	0.263	2.625	Positive	Violet
5	0.131	1.313	Positive	No colour
6	0.066	0.656	Positive	No colour
7	0.033	0.328	Positive	No colour
8	0.016	0.164	Positive	No colour
9	0.008	0.082	Negative	No colour
10	0.004	0.041	Negative	No colour
11	0.002	0.021	Negative	No colour

dexamethasone nor prednisolone. All samples which were adulterated have been summarized in Figure 4.

methanol solvents mean that prednisolone has the same dissolution with one component in the excipients when these solvents were used, while

**Fig 4: Summary of herbal medicines adulterated with corticosteroids**

Discussion

The solvent selection experiment was aimed to get the solvent which can extract best the active ingredient from the dexamethasone and prednisolone tablet. The single spot which was observed from dexamethasone standards indicated that the solvents were isolating a pure dexamethasone from the tablets. This is supported by Rf value for dexamethasone obtained which is 0.54. This was previous achieved by (Limmatvapirat *et al.*, 2012). The two spots were observed from prednisolone tablets in dichloromethane, ethyl acetate,

prednisolone standards prepared in acetonitrile and ethanol solvents showed one spot. No spot observed in tablet dissolved in cyclohexane solvents, this is because the solvent was less polar compared with the targeted corticosteroids. In this regard extraction of active ingredient from prednisolone tablet is effectively done using medium polar solvent and this due to its medium polarity facilitated by its structure.

Despite the fact that after concentrating the extract all solvents used showed one spot in dexamethasone tablet, this experiment revealed that in order to extract only active ingredient

from the tablet methanol should be used. The extraction of dexamethasone with methanol resulted to high yield of the active ingredients compared to other solvents (dichloromethane, ethyl acetate and ethanol). For prednisolone; in order to extract only active ingredient, acetonitrile HPLC grade solvent should be used because the solvent extracted high yield compared to dichloromethane, ethyl acetate and ethanol likely (Li *et al.*, 2012) extracted prednisolone using acetonitrile.

The optimal concentration for visualization reagent is using equal volume of 3 mol/dm³ sodium hydroxide and 0.625 mg/mL of tetrazolium blue chloride solutions. The chosen concentration used small amount of tetrazolium blue solution thus small amount of tetrazolium blue chloride can be enough to visualize several samples. The use of 6 mol/dm³ resulted to TLC plate disintegration and this was proved when 6 mol/dm³ of sodium hydroxide alone was sprayed over TLC plate and still TLC plate disintegrated.

In confirmation of dexamethasone and prednisolone standards using LC-MS/MS, the spectrum does not correspond to the exactly m/z for targeted steroids. This is due to formation of formate adduct which lead to [M+HCOO]⁻ in negative electrospray mode for both dexamethasone and prednisolone (Giaccone *et al.*, 2017). This increased the m/z value for dexamethasone base peak from 393 m/z [M+H]⁺ to 437 m/z of [M+HCOO] and the base peak for prednisolone increased from 361 m/z [M+H]⁺ to 415 m/z of [M+H].

Prednisolone was the most adulterant present (45%) in samples followed dexamethasone (40%) while samples adulterated with both was the least. This could be due to the cheaper price of prednisolone compared to dexamethasone. The unhealthy practice of adding conventional corticosteroids drugs in herbal medicines has also been reported in other countries (Haneef *et al.*, 2013; Ofori-Kwakye *et al.*, 2014; Park *et al.*, 2016; Zhou *et al.*, 2016). This shows that there are some herbal practitioners that misbehave by adding conventional corticosteroids drugs in herbal medicines. The prolonged use of corticosteroid therapy poses risk to consumers including poor control of adrenal axis, herpes

keratitis, Cushing syndrome, pneumonia and peptic ulcers (Ariffin *et al.*, 2021; Garza-ocañas *et al.*, 2013; Park *et al.*, 2016).

Conclusion

The TLC method optimized allowed better detection of the two corticosteroids drugs, dexamethasone and prednisolone mixed in herbal medicines. Therefore, this method can be used for rapid screening of corticosteroids adulterants in herbal medicines prior to further analysis for quantification. The optimized TLC method is limited to qualitative analysis only and cannot be applied for quantitative analysis. However, the use of other sophisticated equipment such as LC-MS/MS is recommended to analyse herbal samples which found to contain adulterants.

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APPENDIX

Herbal medicines samples screened for detection of corticosteroids adulterants

S/N	Sample name	UV 254 nm	Color reaction	Adulterant
1	MWNYDC1	Negative	No colour	Not detected
2	MWNYDC2	Positive	Violet	Prednisolone
3	MWNYDC4	Positive	Violet	Prednisolone
4	MWNYDC10	Negative	No colour	Not detected
5	MWNYDC13	Negative	No colour	Not detected
6	MWNYDC14	Negative	No colour	Not detected
7	MWNYDC16	Negative	No colour	Not detected
8	MWNYDC12	Positive	Violet	Prednisolone
9	MORKLS1	Positive	Violet	Prednisolone
10	MORKLS10	Negative	No colour	Not detected
11	MWNYDC13	Negative	No colour	Not detected
12	MWNYDC20	Negative	No colour	Not detected
13	MORKLS50	Negative	No colour	Not detected
14	MORKLS51	Positive	No colour	Dexamethasone
15	MORKLS55	Negative	No colour	Not detected
16	MORKLS57	Negative	No colour	Not detected
17	MORKLS59	Negative	No colour	Not detected
18	MORKLS60	Negative	No colour	Not detected
19	MORKLS61	Positive	No colour	Prednisolone
20	MORKLS2	Positive	No colour	Dexamethasone
21	MORKLS45	Negative	No colour	Not detected
22	MORKLS46	Negative	No colour	Not detected
23	MORKLS49	Positive	No colour	Prednisolone
24	MORKLS52	Negative	No colour	Not detected
25	MORKLS53	Negative	No colour	Not detected
26	MORKLS54	Negative	No colour	Not detected
27	MORKLS62	Negative	No colour	Not detected
28	MORKLS66	Negative	No colour	Not detected
29	MORKLS67	Negative	No colour	Not detected
30	MORKLS56	Negative	No colour	Not detected
31	ARUMER4	Negative	No colour	Not detected
32	ARUARU1	Negative	No colour	Not detected
33	ARUARU2	Negative	No colour	Not detected
34	ARUARU3	Negative	No colour	Not detected
35	ARUARU5	Negative	No colour	Not detected
36	ARUARU6	Negative	No colour	Not detected
37	ARUARU7	Negative	No colour	Not detected

S/N	Sample name	UV 254 nm	Color reaction	Adulterant
38	ARUARU8	Negative	No colour	Not detected
39	NJMAKD34	Negative	No colour	Not detected
40	NJMAKD3	Negative	No colour	Not detected
41	MORKLS69	Negative	No colour	Not detected
42	MORKLS70	Positive	No colour	Prednisolone
43	MORKLS71	Positive	No colour	Prednisolone
44	MORKLS72	Negative	No colour	Not detected
45	MORKLS73	Negative	No colour	Not detected
46	MORKLS83	Positive	No colour	Dexamethasone
47	MORKLS82	Negative	No colour	Not detected
48	MORKLS75	Negative	No colour	Not detected
49	DAKGMC15	Negative	No colour	Not detected
50	NJMAKD4	Negative	No colour	Not detected
51	MORKLS50	Negative	No colour	Not detected
52	DAKGMC1	Negative	No colour	Not detected
53	DAKGMC3	Negative	No colour	Not detected
54	DAKGMC5	Negative	No colour	Not detected
55	DAKGMC10	Positive	No colour	Dexamethasone
56	DAKGMC17	Negative	No colour	Not detected
57	DAKGMC18	Negative	No colour	Not detected
58	NJMAKD1	Positive	No colour	Prednisolone
59	NJMAKD2	Positive	No colour	Prednisolone
60	NJMAKD33	Negative	No colour	Not detected
61	MWNYDC19	Negative	No colour	Not detected
62	MWNYDC21	Negative	No colour	Not detected
63	MWNYDC11	Negative	No colour	Not detected
64	MWNYDC12	Negative	No colour	Not detected
65	MWNYDC15	Negative	No colour	Not detected
66	MORKLS36	Negative	No colour	Not detected
67	MORKLS37	Positive	Violet	Other corticosteroid
68	MORKLS39	Negative	No colour	Not detected
69	MORKLS43	Positive	No colour	Prednisolone
70	MORKLS44	Positive	No colour	Prednisolone
71	ARUMER8	Negative	No colour	Not detected
72	ARUMER9	Negative	No colour	Not detected
73	ARUMER10	Negative	No colour	Not detected
74	IRIIRI03	Negative	No colour	Not detected
75	IRIIRI04	Negative	No colour	Not detected
76	IRIIRI05	Negative	No colour	Not detected

S/N	Sample name	UV 254 nm	Color reaction	Adulterant
77	IRIIRI06	Negative	No colour	Not detected
78	IRIIRI10	Negative	No colour	Not detected
79	IRIIRI18	Negative	No colour	Not detected
80	IRIIRI19	Positive	Violet	Other corticosteroid
81	MORMVO12	Negative	No colour	Not detected
82	KIMOSM8	Negative	No colour	Not detected
83	KIMOSM11	Negative	No colour	Not detected
84	KIMOSM12	Negative	No colour	Not detected
85	KIMOSM15	Negative	No colour	Not detected
86	KIMOSM16	Negative	No colour	Not detected
87	KIMOSM17	Negative	No colour	Not detected
88	KIMOSM18	Negative	No colour	Not detected
89	KIMOSM26	Negative	No colour	Not detected
90	KIMOSM25	Negative	No colour	Not detected
91	MORMVO11	Negative	No colour	Not detected
92	MORMVO13	Negative	No colour	Not detected
93	MORMVO14	Negative	No colour	Not detected
94	MORMVO15	Negative	No colour	Not detected
95	MORMVO16	Negative	No colour	Not detected
96	MORMVO17	Negative	No colour	Not detected
97	MORMVO18	Negative	No colour	Not detected
98	MORMVO19	Negative	No colour	Not detected
99	MORMVO20	Negative	No colour	Not detected
100	KIMOSM02	Negative	No colour	Not detected
101	KIMOSM01	Negative	No colour	Not detected
102	KIMOSM05	Positive	Violet	Dexamethasone
103	KIMOSM07	Negative	No colour	Not detected
104	KIMOSM09	Negative	No colour	Not detected
105	KIMOSM14	Negative	No colour	Not detected
106	KIMOSM19	Negative	No colour	Not detected
107	KIMOSM20	Negative	No colour	Not detected
108	KIMOSM21	Negative	No colour	Not detected
109	KIMOSM22	Negative	No colour	Not detected
110	KIMOSM23	Negative	No colour	Not detected
111	MORMVO1	Negative	No colour	Not detected
112	MORMVO2	Negative	No colour	Not detected
113	MORMVO3	Negative	No colour	Not detected
114	MORMVO4	Negative	No colour	Not detected
115	MORMVO5	Negative	No colour	Not detected

S/N	Sample name	UV 254 nm	Color reaction	Adulterant
116	MORMVO6	Negative	No colour	Not detected
117	MORMVO7	Negative	No colour	Not detected
118	MORMVO8	Positive	Violet	Other corticosteroid
119	MORMVO9	Negative	No colour	Not detected
120	MORMVO10	Negative	No colour	Not detected
121	KIMOSM3	Negative	No colour	Not detected
122	KIMOSM4	Positive	Violet	Dexamethasone and other corticosteroid
123	KIMOSM6	Negative	No colour	Not detected
124	KIMOSM10	Negative	No colour	Not detected
125	KIMOSM13	Negative	No colour	Not detected
126	DARILA01	Negative	No colour	Not detected
127	DARILA11	Negative	No colour	Not detected
128	DARILA12	Negative	No colour	Not detected
129	DARILA14	Negative	No colour	Not detected
130	KIMOSM24	Negative	No colour	Not detected
131	DARILA15	Negative	No colour	Not detected
132	DARILA16	Negative	No colour	Not detected
133	DARILA18	Negative	No colour	Not detected
134	DARILA19	Negative	No colour	Not detected
135	DARILA21	Negative	No colour	Not detected
136	DARILA23	Negative	No colour	Not detected
137	DARILA24	Positive	No colour	Dexamethasone
138	DARILA25	Negative	No colour	Not detected
139	DARILA26	Negative	No colour	Not detected
140	DARILA27	Negative	No colour	Not detected
141	DARILA32	Negative	No colour	Not detected
142	DARILA34	Negative	No colour	Not detected
143	DARILA35	Positive	No colour	Dexamethasone
144	DARILA36	Negative	No colour	Not detected
145	DARILA37	Negative	No colour	Not detected
146	DARILA38	Negative	No colour	Not detected
147	DARILA39	Negative	No colour	Not detected
148	DARILA40	Positive	No colour	Prednisolone
149	DARILA41	Negative	No colour	Not detected
150	DARILA28	Negative	No colour	Not detected
151	DARILA42	Negative	No colour	Not detected
152	DARILA43	Negative	No colour	Not detected
153	DARILA44	Negative	No colour	Not detected
154	DARILA45	Positive	No colour	Dexamethasone
155	DARILA46	Positive	No colour	Dexamethasone
156	DARILA47	Negative	No colour	Not detected

S/N	Sample name	UV 254 nm	Color reaction	Adulterant
157	DARILA48	Negative	No colour	Not detected
158	DARILA49	Negative	No colour	Not detected
159	DARILA50	Negative	No colour	Not detected
160	DARILA51	Negative	No colour	Not detected
161	DARILA52	Negative	No colour	Not detected
162	DARILA53	Negative	No colour	Not detected
163	DARILA54	Negative	No colour	Not detected
164	DARILA55	Negative	No colour	Not detected
165	DARILA56	Negative	No colour	Not detected
166	DARILA57	Positive	No colour	Prednisolone
167	DARILA58	Negative	No colour	Not detected
168	DARILA59	Positive	No colour	Dexamethasone
169	DARILA60	Negative	No colour	Not detected
170	DARILA61	Negative	No colour	Not detected
171	DARILA31	Negative	No colour	Not detected
172	DARILA62	Negative	No colour	Not detected
173	DARILA66	Negative	No colour	Not detected
174	DARILA69	Negative	No colour	Not detected
175	NJMAKT2	Negative	No colour	Not detected
176	NJMAKT3	Negative	No colour	Not detected
177	NJMAKT5	Negative	No colour	Not detected
178	NJMAKT7	Negative	No colour	Not detected
179	NJMAKT8	Negative	No colour	Not detected
180	NJMAKT9	Negative	No colour	Not detected
181	NJMAKT10	Negative	No colour	Not detected
182	NJMAKT11	Negative	No colour	Not detected
183	NJMAKT12	Negative	No colour	Not detected
184	NJMAKT13	Negative	No colour	Not detected
185	NJMAKT14	Positive	No colour	Prednisolone
186	NJMAKT15	Negative	No colour	Not detected
187	NJMAKT16	Negative	No colour	Not detected
188	NJMAKT18	Negative	No colour	Not detected
189	NJMAKT19	Negative	No colour	Not detected
190	NJMAKT20	Negative	No colour	Not detected
191	NJMAKT21	Negative	No colour	Not detected
192	NJMAKT22	Negative	No colour	Not detected
193	NJMAKT23	Negative	No colour	Not detected
194	NJMAKT25	Positive	Violet	Dexamethasone
195	NJMAKT26	Negative	No colour	Not detected

S/N	Sample name	UV 254 nm	Color reaction	Adulterant
196	NJMAKT27	Negative	No colour	Not detected
197	NJMAKT28	Negative	No colour	Not detected
198	NJMAKT29	Negative	No colour	Not detected
199	NJMAKT30	Negative	No colour	Not detected
200	NJMAKT31	Negative	No colour	Not detected
201	DARILA69	Negative	No colour	Not detected
202	NJMAKT6	Negative	No colour	Not detected
203	NJMAKT35	Positive	No colour	Dexamethasone
204	NJMAKT36	Positive	No colour	Prednisolone
205	NJMAKT37	Negative	No colour	Not detected
206	NJMAKT39	Positive	Violet	Both prednisolone & dexamethasone
207	NJMAKT40	Negative	No colour	Not detected
208	NJMAKT41	Negative	No colour	Not detected
209	NJONJT1	Negative	No colour	Not detected
210	NJONJT2	Negative	No colour	Not detected
211	NJONJT5	Negative	No colour	Not detected
212	NJONJT6	Positive	No colour	Dexamethasone
213	NJONJT7	Negative	No colour	Not detected
214	NJONJT8	Positive	No colour	Prednisolone
215	NJONJT9	Negative	No colour	Not detected
216	NJONJT10	Negative	No colour	Not detected
217	NJONJT12	Negative	No colour	Not detected
218	NJONJT13	Negative	No colour	Not detected
219	NJONJT14	Negative	No colour	Not detected
220	NJONJT15	Negative	No colour	Not detected
221	NJMAKT17	Negative	No colour	Not detected
222	NJONJT19	Negative	No colour	Not detected
223	NJONJT20	Negative	No colour	Not detected
224	NJONJT21	Negative	No colour	Not detected
225	NJONJT22	Negative	No colour	Not detected
226	NJONJT24	Negative	No colour	Not detected
227	NJONJT27	Positive	Violet	Prednisolone and other corticosteroid
228	NJONJT28	Negative	No colour	Not detected
229	NJONJT29	Negative	No colour	Not detected
230	NJONJT30	Negative	No colour	Not detected
231	NJONJT31	Negative	No colour	Not detected
232	NJONJT32	Negative	No colour	Not detected
233	NJONJT33	Positive	No colour	Prednisolone
234	NJONJT34	Negative	No colour	Not detected
235	NJONJT35	Negative	No colour	Not detected
236	NJONJT37	Negative	No colour	Not detected

S/N	Sample name	UV 254 nm	Color reaction	Adulterant
237	NJONJT38	Negative	No colour	Not detected
238	NJONJT39	Negative	No colour	Not detected
239	NJONJT40	Negative	No colour	Not detected
240	NJONJT41	Negative	No colour	Not detected
241	NJONJT44	Negative	No colour	Not detected
242	NJONJT45	Positive	No colour	Prednisolone
243	NJONJT46	Negative	No colour	Not detected
244	MWAILE3	Negative	No colour	Not detected
245	MWAILE4	Negative	No colour	Not detected
246	MWAILE5	Negative	No colour	Not detected
247	MWAILE6	Negative	No colour	Not detected
248	MWAILE7	Negative	No colour	Not detected
249	MWAILE11	Negative	No colour	Not detected
250	MWAILE15	Negative	No colour	Not detected
251	MWAILE14	Negative	No colour	Not detected
252	MWAILE16	Negative	No colour	Not detected
253	MWAILE17	Negative	No colour	Not detected
254	MWAILE19	Negative	No colour	Not detected
255	MWAILE20	Negative	No colour	Not detected
256	MWAILE21	Negative	No colour	Not detected
257	MWAILE22	Negative	No colour	Not detected
258	MWAILE25	Negative	No colour	Not detected
259	MWAILE26	Negative	No colour	Not detected
260	MWAILE28	Negative	No colour	Not detected
261	MWAILE29	Negative	No colour	Not detected
262	MWAILE30	Negative	No colour	Not detected
263	MWAILE34	Positive	No colour	Prednisolone
264	MWAILE35	Negative	No colour	Not detected
265	MWAILE36	Negative	No colour	Not detected
266	MWAILE37	Negative	No colour	Not detected
267	MANBAT1	Negative	No colour	Not detected
268	MANBAT4	Negative	No colour	Not detected
269	MANBAT7	Positive	No colour	Prednisolone
270	MANBAT12	Negative	No colour	Not detected
271	MWAILE13	Negative	No colour	Not detected
272	MANBAT14	Positive	No colour	Dexamethasone
273	MANBAT16	Negative	No colour	Not detected
274	MANBAT19	Positive	No colour	Dexamethasone
275	MANBAT40	Positive	No colour	Dexamethasone

S/N	Sample name	UV 254 nm	Color reaction	Adulterant
276	MANBAT43	Negative	No colour	Not detected
277	MANBAT47	Positive	No colour	Dexamethasone
278	MANBAT49	Positive	No colour	Dexamethasone
279	MANBAT50	Positive	No colour	Dexamethasone
280	MANBAT4	Negative	No colour	Not detected
281	ARUMER3	Negative	No colour	Not detected
282	ARUMER5	Negative	No colour	Not detected
283	ARUMER6	Negative	No colour	Not detected
284	ARUMER11	Negative	No colour	Not detected
285	ARUMER15	Negative	No colour	Not detected
286	ARUMER17	Negative	No colour	Not detected
287	ARUMER18	Positive	Violet	Prednisolone
288	ARUMER19	Negative	No colour	Not detected
289	MANBAT21	Positive	Violet	Prednisolone and other corticosteroid
290	MANBAT23	Negative	No colour	Not detected
291	MANBAT24	Negative	No colour	Not detected
292	MANBAT34	Negative	No colour	Not detected
293	MANBAT35	Negative	No colour	Not detected
294	MANBAT38	Negative	No colour	Not detected
295	MANBAT42	Negative	No colour	Not detected
296	MANBAT44	Positive	Violet	Prednisolone
297	MANBAT48	Negative	No colour	Not detected
298	ARUARU4	Negative	No colour	Not detected
299	ARUARU8	Negative	No colour	Not detected
300	ARUARU7	Positive	Violet	Prednisolone
301	ARUARU11	Negative	No colour	Not detected
302	ARUARU12	Negative	No colour	Not detected
303	ARUARU13	Negative	No colour	Not detected
304	SUAGC1	Negative	No colour	Not detected
305	SUAGC2	Positive	No colour	Prednisolone and other corticosteroid
306	SUAGC3	Negative	No colour	Not detected
307	SUAGC4	Negative	No colour	Not detected
308	SUAGC5	Positive	No colour	Dexamethasone
309	SUAGC8	Positive	Violet	dexamethasone
310	SUAGC9	Positive	No colour	Dexamethasone
311	SUAGC10	Negative	No colour	Not detected
312	SUAGC11	Negative	No colour	Not detected
313	SUAGC13	Positive	No colour	Dexamethasone
314	SUAGC15	Positive	No colour	Prednisolone
315	SUAGC16	Negative	No colour	Not detected
316	SUAGC17	Negative	No colour	Not detected

S/N	Sample name	UV 254 nm	Color reaction	Adulterant
317	SUAGC18	Negative	No colour	Not detected
318	SUAGC20	Positive	No colour	Dexamethasone
319	SUAGC21	Negative	No colour	Not detected
320	SUAGC22	Negative	No colour	Not detected
321	SUAGC23	Negative	No colour	Not detected
322	SUAGC24	Negative	No colour	Not detected
323	SUAGC25	Negative	No colour	Not detected
324	SUAGC27	Positive	No colour	Prednisolone
325	SUAGC28	Positive	No colour	Prednisolone
326	SUAGC29	Positive	Violet	Both prednisolone & dexamethasone
327	SUAGC30	Positive	No colour	Prednisolone
328	SUAGC31	Positive	No colour	Prednisolone
329	SUAGC34	Positive	No colour	Prednisolone
330	SUAGC35	Positive	No colour	Prednisolone
331	SUAGC36	Positive	No colour	Prednisolone
332	SUAGC38	Negative	No colour	Not detected
333	SUAGC39	Negative	No colour	Not detected
334	SUAGC40	Positive	No colour	Dexamethasone
335	SUAGC41	Negative	No colour	Not detected
336	SUAGC42	Negative	No colour	Not detected
337	SUAGC43	Negative	No colour	Not detected
338	SUAGC44	Negative	No colour	Not detected
339	SUAGC45	Negative	No colour	Not detected
340	SUAGC46	Negative	No colour	Not detected
341	SUAGC47	Negative	No colour	Not detected
342	SUAGC48	Negative	No colour	Not detected
343	SUAGC49	Negative	No colour	Not detected
344	SUAGC50	Negative	No colour	Not detected
345	SUAGC51	Negative	No colour	Not detected
346	SUAGC52	Positive	Violet	Dexamethasone
347	SUAGC54	Negative	No colour	Not detected
348	SUAGC55	Negative	No colour	Not detected
349	SUAGC56	Negative	No colour	Not detected
350	SUAGC57	Negative	No colour	Not detected
351	SUAGC58	Negative	No colour	Not detected
352	SUAGC59	Negative	No colour	Not detected
353	SUAGC60	Negative	No colour	Not detected
354	SUAGC62	Negative	No colour	Not detected
355	SUAGC63	Negative	No colour	Not detected
356	SUAGC65	Positive	No colour	Dexamethasone
357	SUAGC66	Negative	No colour	Not detected

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S/N	Sample name	UV 254 nm	Color reaction	Adulterant
358	SUAGC67	Negative	No colour	Not detected
359	SUAGC68	Negative	No colour	Not detected
360	SUAGC69	Negative	No colour	Not detected
361	SUAGC70	Positive	No colour	Dexamethasone
362	SUAGC71	Negative	No colour	Not detected
363	SUAGC72	Negative	No colour	Not detected
364	SUAGC73	Positive	No colour	Dexamethasone
365	SUAGC74	Negative	No colour	Not detected
366	SUAGC75	Positive	No colour	Dexamethasone
367	SUAGC76	Positive	No colour	Dexamethasone
368	SUAGC78	Positive	No colour	Dexamethasone
369	SUAGC80	Positive	No colour	Dexamethasone
370	SUAGC81	Positive	No colour	Both prednisolone & dexamethasone
371	SUAGC83	Positive	No colour	Dexamethasone
372	SUAGC84	Positive	No colour	Dexamethasone
373	SUAGC85	Positive	No colour	Dexamethasone
374	SUAGC86	Positive	No colour	Dexamethasone
375	SUAGC87	Positive	Violet	Dexamethasone
376	SUAGC88	Positive	No colour	Dexamethasone
377	SUAGC89	Positive	No colour	Dexamethasone
378	SUAGC90	Positive	No colour	Dexamethasone
379	SUAGC92	Positive	No colour	Dexamethasone
380	SUAGC93	Positive	No colour	Both prednisolone & dexamethasone
381	SUAGC94	Positive	No colour	Dexamethasone
382	SUAGC95	Positive	No colour	Dexamethasone
383	SUAGC96	Positive	No colour	Dexamethasone
384	SUAGC100	Positive	No colour	Dexamethasone
385	SUAGC101	Positive	No colour	Prednisolone
386	SUAGC102	Negative	No colour	Not detected
387	SUAGC103	Positive	No colour	Prednisolone
388	SUAGC104	Positive	No colour	Prednisolone
389	SUAGC105	Negative	No colour	Not detected
390	SUAGC106	Positive	No colour	Prednisolone
391	SUAGC107	Negative	No colour	Not detected
392	SUAGC108	Positive	No colour	Both prednisolone & dexamethasone
393	SUAGC109	Negative	No colour	Not detected
394	SUAGC110	Positive	No colour	Not detected
395	SUAGC111	Negative	No colour	Not detected
396	SUAGC115	Positive	No colour	Both prednisolone & dexamethasone
397	SUAGC116	Positive	No colour	Both prednisolone & dexamethasone
398	SUAGC117	Positive	No colour	Both prednisolone & dexamethasone

S/N	Sample name	UV 254 nm	Color reaction	Adulterant
399	SUAGC119	Positive	No colour	Both prednisolone & dexamethasone
400	SUAGC120	Negative	No colour	Not detected
401	SUAGC121	Positive	No colour	Both prednisolone & dexamethasone
402	SUAGC118	Negative	No colour	Not detected
403	SUAGC122	Positive	No colour	Prednisolone
404	SUAGC123	Positive	No colour	Prednisolone
405	SUAGC124	Positive	No colour	Prednisolone
406	SUAGC125	Positive	No colour	Prednisolone
407	SUAGC126	Positive	No colour	Prednisolone
408	SUAGC127	Positive	No colour	Prednisolone
409	SUAGC128	Positive	No colour	Prednisolone
410	SUAGC129	Positive	No colour	Prednisolone
411	SUAGC130	Positive	No colour	Prednisolone
412	SUAGC133	Positive	No colour	Prednisolone
413	SUAGC131	Positive	No colour	Prednisolone
414	SUAGC134	Positive	No colour	Prednisolone
415	SUAGC135	Positive	No colour	Prednisolone
416	SUAGC136	Negative	No colour	Not detected
417	SUAGC138	Positive	No colour	Dexamethasone
418	SUAGC139	Negative	No colour	Not detected
419	SUAGC141	Positive	No colour	Dexamethasone
420	SUAGC151	Positive	No colour	Dexamethasone
421	SUAGC242	Positive	No colour	Prednisolone
422	SUAGC137	Positive	No colour	Prednisolone
423	SUAGC250	Positive	No colour	Prednisolone
