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Research Article

Detection of Various Prohibited Substances in Thoroughbred Racehorses at Three Major Racetracks in Türkiye: A Nine-Year Experience

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Abstract

The use of prohibited substances raises significant ethical and global issues for horseracing. To mitigate these problems and maintain control, prohibited substance controls are conducted on samples taken from thoroughbred horses before and after races. However, limited information is available on prohibited substances detected as a result of these controls. In this study, 39.935 samples from races held at three racetracks in Türkiye between 2015 and 2023 were analysed qualitatively and/or quantitatively using gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, high-resolution mass spectrometry, inductively coupled plasma mass spectrometry, and a biochemistry autoanalyser. Overall, 219 violations caused by 314 findings were reported. Of these findings, 140 were International Screening Limit, 86 were prohibited for use at any time, 60 were threshold, and 28 were substances within the scope of International Residue Limit. Consequently, violations detected at three major racetracks in Türkiye over nine years were evaluated, and useful information was shared with scientists working in this field, race veterinarians, horse owners, and trainers.

Keywords: Horseracing, anti-doping, Türkiye, prohibited substance, racetracks

Introduction

Doping in racehorses is described as the administration of any substance, other than normal food, intended to alter a horse's speed, strength, or stamina in running. In horse sports, prohibited substances not only include doping agents used to enhance performance but also legitimate therapeutic agents (off-label use of veterinary or human drugs necessary for the horse's health) that may indirectly influence performance. For this reason, regulatory rules have been developed by the International Federation of Horseracing Authorities (IFHA) to control the substances used in horseracing. Türkiye has adopted and applies the rules set by the IFHA without exception [1].

The prohibited substances in horseracing and their exceptions are defined in Article 6A of the "International Agreement on Breeding, Racing and Wagering (IABRW)" published by IFHA [1]. Accordingly, substances capable of acting on one or more of the cardiovascular, respiratory, digestive, urinary, reproductive, musculoskeletal, hematologic, immune (except licensed vaccines against infectious agents), and endocrine systems in mammals, as well as their synthetic counterparts, masking agents, oxygen carriers, and substances that directly or indirectly affect or manipulate gene expression, are classified as prohibited substances. These are divided into four categories: substances subject to International Screening Limits (ISL) [2-4], substances with threshold values [1], international residue limited (IRL) substances [5], and substances that are prohibited for use at any time [1].

In this context, the urine and plasma limits of ISL substances permitted for therapeutic use by the IFHA was defined, and the rules for their use were established. When a substance exceeding the ISL is detected during screening analyzes for the control of prohibited substances, a qualitative confirmatory analysis, usually performed by mass spectrometry is carried out to confirm the presence of the prohibited substance. Quantification

is not required for this confirmation. In addition, analyzes using different types of mass spectrometers (screening and confirmation) based detection of the parent compounds or metabolites. Under these rules, the detection of a substance above the ISL constitutes a direct violation. Even if substances are detected below the ISL, their presence in combination with another substance sharing the same mechanism of action or with a masking agent also constitutes a violation [3,4].

Endogenous substances in horses (e.g., testosterone, carbon dioxide) or substances present in plants traditionally grazed or used as horse feed (e.g., arsenic, cobalt) are defined under the category of threshold substances. During screening analyzes, if a substance in this group is suspected of exceeding the threshold, quantitative analyzes are performed. If exceedance is confirmed, it is reported as a violation [1,6,7].

Internationally harmonized residue limits are applied to control certain feed contaminants and environmental substances. The substances in this category are controlled at the screening level, like those of ISL substances [5].

Detection of these substances themselves, their metabolites, isomers, metabolite of the metabolites, or prodrug forms, as well as any scientific evidence indicating their administration, causes a violation [1].

Anti-doping laboratories generally analyze prohibited substances in accordance with IFHA rules and the technical criteria established by the Association of Official Racing Chemists (AORC) [8-10]. However, published reports on the findings of these laboratories are quite limited. The most comprehensive report on the use of prohibited substances in racing covers the 12 years [11]. IFHA has published the results of all laboratories for specific periods [12]. These reported violation rates vary among studies: in Illinois, a violation rate of 0.45% was observed over a five-year period [13], in Louisiana, 1.01% of 52.909 samples analysed [14], in Iran, 31.4% of 656 samples analysed [15,16], in Cyprus, 161 violations (1.52%) were reported as a result of analyzing 94.800 samples [17], in the Czech Republic, twelve different prohibited substances were detected, alone or in combination, in 2.03% of 641 samples [18], and in Italy 549 violations (0.4%) were reported as a result of analyzing 104.770 samples [19]. Furthermore, the results of similar studies are evaluated in another study [20].

This study aimed to retrospectively analyze prohibited substance findings in urine and blood samples collected from three racetracks in Izmir, Bursa, and Kocaeli, Türkiye, between 2015 and 2023, and to provide information for scientists, racing veterinarians, horse owners, and trainers.

Material and Methods

Ethics Statement

This study does not require ethics committee approval, as it is based on previously published analysis results.

The Laboratory and Samples

Urine and blood samples collected before and after thoroughbred horse races held at the racetracks in Izmir, Bursa, and Kocaeli were divided into two groups: "A" and "B." The portion of each sample designated as the "A sample" was sent to the Istanbul Pendik Veterinary Control Institute Doping Laboratory, while the "B sample" was retained at the respective racetrack for confirmatory analysis.

Each sample underwent an initial screening analysis, followed by confirmatory testing for those in which the presence of prohibited substances was suspected. If the presence of a prohibited substance was confirmed, the finding was reported as a positive result. Following such notifications, the corresponding "B sample" was transferred by the relevant racecourse to another authorized doping laboratory (Veterinary Control Central Research Institute Doping Laboratory, Ankara) to confirm the presence of the prohibited substance.

Samples received at the laboratory were analysed according to established procedures [21,22] within 1-15 days of receipt. Negative samples were stored for one month, while positive samples for six months.

Instrumentation and Methodology

Analysis of prohibited substances in blood and urine samples were carried out in accordance with the guidelines of the Association of Official Racing Chemists (AORC) [8], IFHA [1], Commission Implementing Regulation (EU) 2021/808 and ISO/IEC 17025:2017 standards. Mass spectrometer calibrations (in both positive and negative ion modes) and instrument cleaning were performed for each sample batch before analysis. After calibration and cleaning, a system suitability test was conducted to verify the performance and stability of the instrument before beginning the analytical sequence.

Urine Samples Analysis

Urine samples collected post-race and submitted to the laboratory were divided and subjected to both enzymatic and alkaline hydrolysis. A 15 mL aliquot was taken for enzymatic hydrolysis and subsequently divided into three portions. Each portion was extracted using different solid-phase extraction (SPE) procedures. The resulting extracts were individually analysed on mass spectrometric instruments selected according to the chemical characteristics of the compounds (LC-MS/MS [23-25],

UHPLC-HRMS [26] and GC-MS [2,27,28]). The portion subjected to alkaline hydrolysis was extracted using a SPE method and then analysed by GC-MS [2]. Arsenic and cobalt were analysed by inductively coupled plasma mass spectrometry (ICP-MS) after acidic dilution (1:25, v/v) of the samples [29]. Another portion of the urine sample was diluted 1:5 (v/v) without hydrolysis, ultracentrifuged, and analysed using UHPLC-HRMS [30].

Blood Samples Analysis

The plasma was separated from the blood samples and divided into five portions. Acidic hydrolysis was performed on two of these portions. After hydrolysis, extractions using different types of SPE cartridges (C18 and mixed-mode cation exchange) were performed, and the resulting extracts were analysed by LC-MS/MS [23], and UHPLC-HRMS. Another portion underwent SPE extraction (C18) without hydrolysis. Another portion was diluted 1:5 (v/v), centrifuged, and analysed by UHPLC-HRMS [30]. The final portion, used for arsenic and cobalt determination, was diluted 1:100 (v/v) and analysed by ICP-MS [29]. Blood samples collected prior to the race were analysed using a biochemistry autoanalyzer to determine total carbon dioxide (TCO_2) concentrations.

All findings, factors, and notable analysis results identified over 9 years are demonstrated in the *Table 1*, *Table 2*, *Table 3*, and *Table 4*.

Results

Between 2015 and 2023, a total of 39.935 samples (2.110 blood samples, including 220 pre-race samples, and 37.825 urine samples) taken from thoroughbred horses and sent to the laboratory were analysed for the presence of prohibited substances. As a result of the analysis, 219 violations were reported (0.55% of all samples). Of these findings, 66 cases (0.2%) involved multiple-substance violations, whereas 153 cases (0.4%) involved a single-substance. Of multiple-substance violations, 24 were detected in samples from Izmir, 19 from Bursa, and 23 from Kocaeli racetracks. In contrast, single-substance violations were detected in 40 samples from Kocaeli, 50 from Bursa, and 63 from Izmir racetracks *(Table 1)*.

The highest annual violation rate during the nine-year study period was recorded in 2015 (2.09%), while the lowest was in 2018 (0.34%). Relative to the total number of samples analysed, violations were detected in 0.65% of samples from Kocaeli (63 from 9.633 samples), 0.56% from Bursa (69 from 12.247 samples), and 0.48% from Izmir (87 from 18.055 samples) *(Fig.1)* *(Table 1)*.

Of the 314 findings that resulted in violations during this study, 45 different prohibited substances were detected. According to IFHA prohibited substance classification, 140

Table 1. *Distribution of samples according to the racetracks from which they originate and violation rates*

Racetrack		2015			2016			2017			2018			2019			2020			2021			2022			2023			9 Year Total		
	Number of samples [a]	342	413	250	1451	901	3150	5502	832	807	1403	3042	1290	1806	2230	5326	1280	1630	2005	4915	1138	1481	1724	4343	1154	1811	2385	5350	1085	1716	2469	5270	1061	1682	2439	5182	9633	12247	18055	39935
	Single substances[b]	5	6	6	10	1	7	18	3	9	14	26	6	4	3	13	4	5	9	18	5	10	10	25	2	5	9	16	2	9	4	15	3	1	1	5	40	50	63	153
	Multiple substances[c]	0	2	2	3	0	2	5	2	1	2	5	0	1	0	5	1	2	1	4	2	5	9	16	0	2	4	6	4	5	6	15	3	1	1	5	23	19	24	66
	% Total violations[d]	1.46	1.94	3.2	0.9	0.11	0.29	0.42	0.60	1.23	1.14	1.01	0.78	0.28	0.13	0.34	0.39	0.43	0.5	0.45	0.97		0.58	0.71	0.17	0.39	0.55	0.41	0.55	0.82	0.41	0.57	0.57	0.42	0.33	0.41	0.65	0.56	0.48	0.55
Kocaeli		21			23			31			18			22			31			22			30			21			219		
Bursa																														
Izmir																														
TOTAL																														
Total Violations																														

[a] The number of samples analysed, [b] The number of single-substance violations, [c] The number of multiple-substance violations, [d] The % percentage of violation received according to the number of samples taken

Table 2. *Distribution of detected findings between 2015 and 2023 according to IFHA groups, mechanisms of action, and quantities of single and multiple substance findings*

Name	Effect	Distribution of Single and Multiple Substance Findings by Years																		Number of Finding
		2015		2016		2017		2018		2019		2020		2021		2022		2023		
Substances prohibited for use at any time		**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	
Altrenogest	Oestrus suppression									1										1
Capsaicin	Topical analgesic														2					2
Cocaine	Stimulant													1				1		2
Diisopropylamine	Vasodilator									3		5								8
Dyphylline	Muscle relaxant	2	1	1								1	2	1	1	1				10
Etodolac	NSAID														1	2	2	1		6
Etofenamat/Flufenamic acid	NSAID	1	1					1				2				2	4			11
Heptaminol	Stimulant											1								1
Levamisole	Stimulant		2				2												1	5
Neostigmine	Anticholinesterase	1																		1
Nikethamide	Stimulant	1	1										1							3
Pemoline	Stimulant	1	2																	3
Pentoxifylline	Vasodilator							2		1		2		1						6
Procaine	Local anaesthetic			2	1		2	1	2		1	1		2						12
Pyrilamine	Antihistamine			1													2			3
Ranitidine	Histamine-2 blocker				1		1			1			2			1				6
Sildenafil	Vasodilator													1		1				2
Tenoxicam	NSAID											1			1					2
Trenbolone	Anabolic Steroid															1				1
Verapamil	Antiarrhythmic					1														1
Total																				**86**
Threshold substances		**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	
Arsenic	Stimulant/toxic					15		2				3		4		4		2		30
Cobalt	Erythropoiesis					8	1	3		7		3		2	1	2	1	1	1	30
Total																				**60**
International screening limited substances		**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	
Ambroxol	Mucolytic								2				1				2			5
Betamethasone	Corticosteroid								1	2	1		1	1						6
Clenbuterol	Bronchodilator													1						1
Dexamethasone	Corticosteroid						1						1			1			2	5
Diclofenac	NSAID						1				1									2
Flunixin	NSAID	1		1	1	2	3		1	1					1		5		13	29
Furosemide	Diuretic	1																		1
HEPS	Sedative									1	1									2
Hydroxy lidocaine	Local anaesthetic								1		1		1		1	1	2		3	10
Hydroxy xylazine	Sedative							2											3	5
Ketoprofen	NSAID	2			2		1						1		1		2		1	10

Table 2. Continue

Name	Effect	Distribution of Single and Multiple Substance Findings by Years																		Number of Finding
		2015		2016		2017		2018		2019		2020		2021		2022		2023		
International screening limited substances		**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	
Meloxicam/Hydroxymethyl meloxicam	NSAID												2		2		7		1	12
Methocarbamol*	Muscle relaxant	1					1							1					3	6
Methylaminoantipyrine	NSAID	3		1	3		1		2	1	1		4		2		7		4	29
Naproxen	NSAID	1		9																10
N-butyl scopolamine	Parasympathetic	1											1		1					3
Phenylbutazone	NSAID										1									1
Triamcinolone acetonide	Corticosteroid														1		2			3
Total																				**140**
Residue Limits Substances		**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	**S**	**M**	
Atropin	Anticholinergic		1		1		1		1											4
Caffeine	Stimulant	1	1	4				1	2			1				1			3	14
Morphine	Opiod analgesic					1					1	5		3						10
Total																				**28**
General total																				**314**

Ambroxol: Metabolites of bromhexine, HEPS: Metabolites of acepromazine, Hydroxy lidocaine: Metabolites of lidocaine, Hydroxymethyl meloxicam: Metabolites of meloxicam, Hydroxy xylazine: Metabolites of xylazine, Methylamino antipyrine: Metabolites of dipyrone, Flufenamic acid: Metabolites of etofenamate
** Asian screening limited. S: Single Substance. M: Multiple Substance*

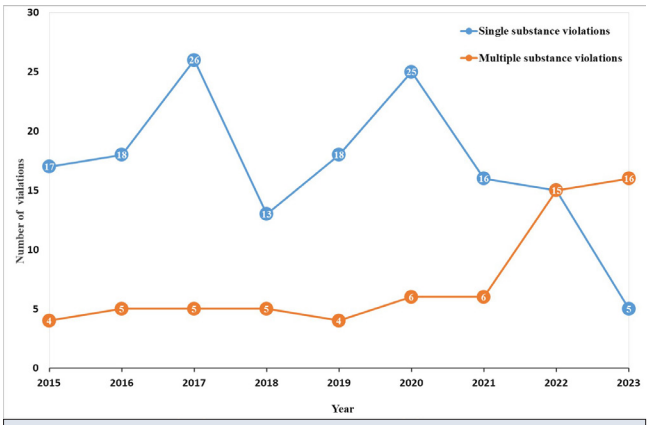

Fig 1. Distribution of violations due to multiple/single findings by years

findings were ISL, 86 were "substance prohibited for use at any time," 60 were "threshold substances," and 28 were within the scope of the IRL. Among these substances, 111 were non-steroidal anti-inflammatory drugs (NSAIDs), 58 were stimulants, 22 were local anaesthetics, 16 were vasodilators, 14 were corticosteroids, and 10 had opioid analgesic effects *(Table 2)*.

According to the results, arsenic and cobalt were the most frequently detected substances within the threshold substance category, each detected in 30 cases. All arsenic violations were caused by single-substance findings, whereas 4 of the cobalt cases involved multiple-substance violations; in the remaining cases, cobalt was detected alone *(Table 2)*.

In the present study, all of the 28 violation findings caused by the substances covered by the IRL were due to atropine, caffeine, and morphine. Seventeen of these findings were caused by single-substance violations, while the others were multiple-substance violations. In this category, caffeine caused the most violations with 14 findings, followed by morphine with 10 findings and atropine with 4 findings *(Table 2)*.

Fifty-two of these findings caused multiple-substance violations, while 34 caused single-substance violations. These violations were mostly caused by NSAID (etofenamate/flufenamic acid 11 finding, etodolac 6 finding), local anaesthetic (procaine 12 finding), muscle relaxant (diphylline 10 finding), and vasodilator (diisopropylamine 8 finding) effective substances *(Table 2)*.

Thirty-five of the ISL substance findings resulted in single-substance violations, and the others (105 findings) resulted in multiple-substance violations *(Table 1)*. Some of these identified violations resulted from the detection of multiple prohibited substances in combination, while others resulted from the identification of a single prohibited substance. Due to the continuous changes in substance/formulation findings detected in multiple-substance

Table 3. *Distribution of multiple substance violations involving procaine, meloxicam and flunixin by year*

Multiple Substance Violations Containing Procaine			**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**	**2023**	**Total**
Multiple Substance Violations Containing Meloxicam and Metabolites												
Procaine	+	Methylaminoantipyrine, ketoprofen		1								**1**
		Ranitidine		1								**1**
		Methocarbamol, flunixin			1							**1**
		Ketoprofen			1							**1**
		Flunixin, atropin				1						**1**
		Methylaminoantipyrine, flunixin				1						**1**
		Methylaminoantipyrine					1					**1**
		Ranitidine, hydroxy lidocaine, methocarbamol						1				**1**
Total				**2**	**2**	**2**	**1**	**1**				**8**
Hydroxymethyl meloxicam/ Meloxicam	+	Methylaminoantipyrine, ambroxol						1				**1**
		Nikethamide, dyphylline, methylaminoantipyrine						1				**1**
		Etodolac, capsaicine							1			**1**
		Flunixin, ambroxol								1		**1**
		Flunixin, methylaminoantipyrine								1		**1**
		Flunixin, triamcinolone acetonide								1		**1**
		Etofenamate, cobalt								1		**1**
		Hydroxy lidocaine, methylaminoantipyrine								1		**1**
		Methylaminoantipyrine, flunixin								1		**1**
		Dexamethasone, levamisole									1	**1**
		Ketoprofen, meloxicam, methylaminoantipyrine								1	1	**2**
Total								**2**	**1**	**7**	**2**	**12**
Multiple substance violations containing flunixin												
Flunixin	+	Hydroxy lidocaine									2	**2**
		Cobalt									1	**1**
		Dexamethasone									1	**1**
		Diclofenac, caffeine				1						**1**
		Caffeine									1	**1**
		Hydroxymethyl meloxicam, ambroxol								1		**1**
		Hydroxymethyl meloxicam, methylaminoantipyrine								1		**1**
		Hydroxymethyl meloxicam, triamcinolone acetonide								1		**1**
		Hydroxy xylazine									3	**3**
		Methocarbamol									2	**2**
		Methocarbamol, methylaminoantipyrine									1	**1**
		Methocarbamol, procaine			1							**1**
		Methylaminoantipyrine								1	2	**3**
		Methylaminoantipyrine, hydroxymethyl meloxicam								1		**1**
		Procaine, atropine				1						**1**
		Procaine, methylaminoantipyrine				1						**1**
Total			**0**	**0**	**1**	**3**	**0**	**0**	**0**	**5**	**13**	**22**

Table 4. *Distribution by year of some painkillers and NSAIDs resulting in single-substance violations*

Substances	2015	2016	2017	2018	2019	2020	2021	2022	2023
Betamethasone/dexamethasone					2		1		
Etodolac								2	
Tenoxicam						1			
Hydroxy xylazine				2					
Morphine			1			5	3		
Etofenamate/flufenamic acid	1			1		2		2	
Flunixin	1	1	2		1				
Ketoprofen	2								
Methylaminoantipyrine	3	1			1				
Naproxen	1	9							

violations, such violations were generally identified once. However, the combinations of flunixin + hydroxy lidocaine and flunixin + methocarbamol were each detected twice, whereas the flunixin + hydroxyxylazine and flunixin + methylaminoantipyrine combination was detected three times. However, significant changes over time were detected in both multiple and single-substance findings *(Table 3, Table 4)*.

Discussion

When interpreting the results, it is worth noting that the Pendik Veterinary Control Institute Doping Laboratory started its activities in October 2015; racetracks were closed for 3 months because of the suspension of horseracing in 2020 due to the Covid-19 pandemic, and elemental analysis of horse urine and blood began in 2017. According to the findings, the violation rate was calculated as 0.55% in 39.935 samples. This rate is lower than those reported from racetracks in Iran [15], the Czech Republic [18], Cyprus [17], and Louisiana (USA) [14], but higher than those reported from racetracks in Illinois (USA) [13] and Italy [19]. These differences are thought to be associated with national regulatory frameworks, analytical capacity of laboratories, regional differences in food sources, and substance use practices.

In this current study, all detected threshold substance violations were caused by arsenic and cobalt *(Table 2)*. Arsenic has been used as a tonic in horses and is considered a potent doping agent due to its potential performance-enhancing properties [6,31]. Cobalt, a well-known chemical inducer of hypoxia-like responses, has been utilised clinically to stimulate erythropoiesis in patients with chronic anaemia and to promote physiological adaptation to low-oxygen conditions [7,32]. Until 2015, licensed formulations containing arsenic were available in several countries; however, their approval was subsequently suspended worldwide because of carcinogenic effects [29]. Despite this, arsenic has been reported to occur in homoeopathic products and various plant sources [29,33]. In contrast, there are cobalt-containing approval formulations worldwide; moreover, cobalt may also be present as a component of premixes used in horse nutrition [7].

Nevertheless, arsenic and cobalt violations have been reported in thoroughbred racehorses [29,34]. Consistent with previous studies, the results of the present study indicate that, following 15 arsenic violations in 2017, the number of detections declined in subsequent years; however, the continued detection of arsenic in 2018 (2 violations), 2020 (3 violations), and 2021-2022 (4 violations) suggests that this substance may have been administered either illegally or unknowingly.

Similarly, cobalt accounted for a high number of violations in 2017 (8 violations), concomitant with arsenic, and despite a reduction in arsenic detections, cobalt violations increased again in 2019 (7 violations) before declining from 2021 onwards *(Table 2)*. This pattern suggests that during the period in which arsenic violations decreased (2018-2019), there may have been a shift towards alternative substances with erythropoietic properties, such as cobalt.

Furthermore, the frequent detection of arsenic and cobalt violations within short time intervals during specific years suggests that racehorses may have been feeds or formulations with unknown composition may be responsible. This detection indicates that threshold substance violations may arise not only from intentional administration but also from indirect or inadvertent exposure pathways. In addition, the detection of a high number of violations in 2017, when the laboratory first began arsenic and cobalt analyses, can be considered an important indicator that substances not included in the

analytical scope may have been widely used *(Table 2)*.

Diisopropylamine (DIPA) is classified as a substance that has always been prohibited for use in racehorses [1]. Known to exhibit physiological effects similar to those of cobalt, DIPA was first detected in this study in 2019, with 3 violations identified in 2019 and 5 in 2020 [35]. Notably, during the period in which DIPA was detected, the frequency of cobalt violations decreased compared with previous years (7 violations in 2019 and 2 in 2020). This detection suggests that DIPA may have been preferred as an alternative to cobalt use *(Table 2)*.

In residue limited substances category, caffeine was the most frequently detected substance (14 findings), followed by morphine (10 findings) and atropine (4 findings) *(Table 2)*. Caffeine is a substance that has stimulant effects on the central nervous system and the musculoskeletal system. In addition, it has mild analgesic, bronchodilator/vasodilator, and diuretic properties [36]. Morphine, a substance derived from *Papaver somniferum*, is widely used in horses as a narcotic analgesic and anaesthetic [37]. Atropine is defined as a prototypical muscarinic receptor antagonist [38]. Because these substances may occur at variable concentrations in feeds used for horse nutrition [37-41], IFHA has established substance-specific IRLs for blood and urine [5]. In addition, licensed pharmaceutical formulations containing each of these substances are available in various countries, indicating multiple potential sources of exposure.

In this study, the detection of morphine (8 violations between 2020 and 2021) within a specific time period, followed by the cessation of these violations, suggests that a particular formulation may have been administered. However, the proximity of Izmir and Bursa to Afyon, where legal poppy *(Papaver somniferum)* cultivation is carried out, together with the presence of well-developed livestock and feed supply infrastructures in these provinces, also suggests the possibility of feed-related exposure [42]. Within this study, the detection of five morphine findings at the Bursa racetrack and four at the Izmir racetrack supports this interpretation.

Similarly, caffeine was detected as a single substance in some cases and in combination with analgesics and muscle relaxants in others, while atropine was detected exclusively in multiple-substance violations *(Table 2)*.

These findings suggest that some of the detected substances may be associated with indirect or inadvertent exposure through feed. Nevertheless, based on the available analytical data, it is not possible to definitively distinguish whether the detected IRL substances, including caffeine, atropine, and morphine, originated from nutritional sources or from deliberate pharmaceutical administration.

As in previous studies, the most common reason for violations in this study was the items within the scope of ISL [20]. One of the main reasons for single-substance violations within the scope of the ISL may be the racing of horses under treatment without adherence to the detection times established by the IFHA [43]. Another possible reason could be that doping laboratories do not report findings below the ISL. This practice can create the idea that these substances cannot be detected by laboratories, thus leading to uncontrolled drug use. On the other hand, horse owners and veterinarians may not be sufficiently informed about the detection times of drugs [43].

In multiple-substance violations where metamizole and flunixin were detected, low concentrations of local anaesthetics, corticosteroids, and other NSAIDs were also detected. The fact that the detection times [43] of these detected substances are very close to each other can be considered as an indication that substances were administered at the same time, and it can also be considered as an indicator of the possibility of some non-defined cocktail formulations being used.

The majority of violations involving substances prohibited for use at any time were associated with NSAIDs (etofenamate, flufenamic acid, and etodolac), local anaesthetics (procaine), bronchodilators (dyphylline), and vasodilators (DIPA). Although rare, substances not approved for veterinary use, including verapamil, sildenafil, cocaine, and neostigmine, were also detected. In addition, nikethamide in 2015, verapamil in 2018, heptaminol in 2020, and cocaine in 2021 were detected only once. Similarly, findings of DIPA, pentoxifylline, and pyrilamine were observed during specific periods but were not detected afterwards *(Table 2)*. These findings may suggest a tendency to favour alternative compounds believed to be undetectable, instead of substances already detected by the laboratory.

In this present study, etodolac and dyphylline, which have very short detection times, caused violations by being detected at low concentrations on 6 and 10 occasions, respectively [44,45]. While three etodolac findings were detected as single-substance violations and three as multiple-substance violations, five dyphylline findings were identified as single-substance violations, and the remaining five as multiple-substance violations in combination with NSAIDs *(Table 2)*. These results suggest that substances with short detection times may have been administered in a manner consistent with their pharmacokinetic properties.

Our findings reveal that single and multiple-substance violations vary over the years and that, particularly from 2019 onwards, there has been a marked and sustained increase in multiple-substance violations *(Fig. 1)*. This

situation suggests not only an increase in the frequency of violations but also a structural transformation in the nature.

Within the scope of the study, certain multiple-substance findings were detected more frequently during specific periods. Multiple-substance violations involving procaine, which began to be detected in 2016, had almost ceased by the end of 2020. Following the cessation of these violations, the detection of combinations containing meloxicam/hydroxymeloxicam began, and after these findings ended in 2022, multiple-substance violations containing flunixin started to be detected *(Table 3)*. This data suggests that users may have shifted to alternative substances or formulations to avoid previously detected compounds. These findings indicate that multiple-substance violations are not random but rather that specific substance combinations were deliberately used or prepared. Although multiple-substance violations have been reported in previous studies, the temporal changes and substance profiles observed in this study indicate, unlike the existing literature, the presence of a more systematic approach to multiple-substance use [16,20].

Findings related to single-substance violations indicate that the detection profile of certain NSAIDs and analgesic agents has changed over time. Until 2018, these violations were caused by etofenamate/flufenamic acid, ketoprofen, methylaminoantipyrine, and naproxen; after 2018, they were more frequently caused by betamethasone/dexamethasone, etodolac, etofenamate/flufenamic acid, hydroxylidocaine, morphine, and tenoxicam *(Table 4)*. These findings indicate a significant shift in the use of NSAID/analgesic prohibited substances from 2018 onwards.

In conclusion, this nine-year retrospective evaluation provides a comprehensive overview of prohibited substance findings in thoroughbred racehorses in Türkiye. The results demonstrate that both single and multiple-substance violations persist over time, with a notable increase in multiple-substance detections in recent years. Temporal changes in detected substances emphasise the importance of ongoing monitoring approaches, harmonised regulatory thresholds, and increased awareness of potential feed-related contamination. These findings contribute region-specific data to the international literature and support ongoing efforts to ensure fair competition and protect horse welfare.

Declarations

Availability of Data and Materials: The data that support the findings of this study are available from the corresponding author (E. Kabil) upon reasonable request.

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References

1. **IFHA:** International agreement on breeding, racing and wagering and appendixes. **In,** Int. Agreem. 2022. https://www.ifhaonline.org/Default.asp?section=IABRW&area=15; *Accessed:* 17 Nov 2024.
2. **Göktaş EF, Kabil E, Arıöz F:** Quantification and validation of nine nonsteroidal anti-inflammatory drugs in equine urine using gas chromatography-mass spectrometry for doping control. *Drug Test Anal,* 12, 1065-1077, 2020. DOI: 10.1002/dta.2864
3. **IFHA:** International screening limits-plasma definition and recommendations for their application in the control of therapeutic substances. 2025. **In,** Int. Screen. Limits-Plasma. https://www.ifhaonline.org/Default.asp?section=IABRW&area=6; *Accessed:* 17 Nov 2024.
4. **IFHA:** International screening limits-urine definition and recommendations for their application in the control of therapeutic substances. 2022. **In,** Int. Screen. Limits-Urine. https://www.ifhaonline.org/default.asp?section=iabrw&area=1; *Accessed:* 17 Nov 2024.
5. **IFHA:** Recommendations for control of feed contaminants and environmental Substances background. **In,** Residue Limits - Urine Plasma. 2022. https://www.ifhaonline.org/Default.asp?section=IABRW&area=18; *Accessed:* 17 Nov 2024.
6. **Chan GHM, Tang W, Curl P, Lin Y, Wan TSM, Ho ENM:** Doping control analysis of total arsenic in equine plasma. *Drug Test Anal,* 12, 1462-1469, 2020. DOI: 10.1002/dta.2896
7. **Ho ENM, Chan GHM, Wan TSM, Curl P, Riggs CM, Hurley MJ, Sykes D:** Controlling the misuse of cobalt in horses. *Drug Test Anal,* 7, 21-30, 2015. DOI: 10.1002/dta.1719
8. **AORC:** AORC Guidelines for the Minimum Criteria for Identification by Chromatography and Mass Spectrometry. 2024. https://www.aorc-online.org/documents/aorc-ms-criteria-ilacg7/; *Accessed:* 18 Nov 2024.
9. **AORC:** Guidelines for referee analysis. 2008. https://www.aorc-online.org/documents/guidelines-for-referee-analysis/; *Accessed:* 18 Nov 2024.
10. **IFHA:** Performance specification of the laboratories for doping control required by the International Federation of horseracing authorities. 2019. **In,** Perform. Specif. Lab. https://www.ifhaonline.org/Default.asp?section=IABRW&area=7; *Accessed:* 18 Nov 2024.
11. **Moss M S:** Survey of positive results from racecourse antidoping samples received at racecourse security services laboratories. *Equine Vet J,* 16, 39-42, 1984. DOI: 10.1111/j.2042-3306.1984.tb01846.x
12. **IFHA:** Advisory council on equine prohibited substances and practices. **In,** Advis. Counc. 2022. https://www.ifhaonline.org/default.asp?section=About%20IFHA&area=25; *Accessed:* 18 Nov 2024.
13. **Taddei L, Benoit M, Sukta A, Peterson J, Gaensslen RE, Negrusz A:** Detection of various performance enhancing substances in specimens collected from race horses in Illinois: A five year experience. *J Anal Toxicol,* 35, 438-443, 2011. DOI: 10.1093/anatox/35.7.438
14. **Waller P, Lomnicka I, Lucas C, Johnson S, Dirikolu L:** The medication violations in racehorses at Louisiana racetracks from 2016 to 2020. *Vet Med Sci,* 8, 553-560, 2022. DOI: 10.1002/vms3.724

15. **Lotfollahzadeh S, Mokhber-Dezfouli MR, Tajik P, Bokaie S, Watson DG:** A survey on two years of medication regulation in horse races in Iran: A survey on the two years medication regulation in horse races in Iran. *Equine Vet J,* 42, 161-163, 2010. DOI: 10.2746/042516409X471449
16. **Tehrani-Sharif M, Zakian A:** Evaluation of different patterns of drug consumption in the ranking of race horses in national racing of Iran: A retrospective study 2002-2015. *Farm Anim Health Nutr,* 1, 39-45 2022. DOI: 10.58803/fahn.v1i2.11
17. **Spanoudes K, Diakakis N:** Equine doping: Perceptions, adverse effects and Cyprus hippodrome case review for the years 2001-2010. *J Hell Vet Med Soc,* 66:15, 2017. DOI: 10.12681/jhvms.15578
18. **Hytychova T, Dosedelova K, Jahn P:** Doping control in horses in the Czech Republic in 2010-2019. *Acta Vet Brno,* 92, 39-46, 2023. DOI: 10.2754/avb202392010039
19. **Roccaro M, Rinnovati R, Stucchi L, La Rocca F, Cascio G, Peli A:** Survey on 9 years of anti-doping controls in horse races in Italy. *Equine Vet J,* 57, 1592-1599, 2025. DOI: 10.1111/evj.14496
20. **Moreira F, Carmo H, Guedes de Pinho P, Bastos M de L:** Doping detection in animals: A review of analytical methodologies published from 1990 to 2019. *Drug Test Anal,* 13, 474-504, 2022. DOI: 10.1002/dta.2999
21. **Göktaş EF, Kabil E, Güneş E, Korkmaz S, Kadioğlu TA, Cücü AK:** Determination and stability of some international screening limited drugs in equine blood by LC-MS/MS. *Anarak Univ Vet Fak Derg,* 66, 363-372, 2019. DOI: 10.33988/auvfd.538782
22. **Göktaş EF, Kabil E:** Stability of nonsteroidal anti-inflammatory drugs in urine and solution: Effects of degradation on analytical assessment. *Bioanalysis,* 15, 407-419, 2023. DOI: 10.4155/bio-2023-0029
23. **Göktaş EF:** Yarış atlarının idrar örneklerinde bazı ilaç etkin maddelerinin eşzamanlı analizleri için sıvı kromatografisi-kütle spektrometresi ve/veya gaz kromatografisi-kütle spektrometresi Ile metot geliştirilmesi ve analitik metot validasyonu. *PhD Thesis.* Marmara University Institute of Health Sciences. 2019.
24. **Kabil E, Göktaş EF, Güneş E, Yatanaslan L, Zor TA, Tektaş MH, Inceman B, Tufan M:** Cocktail drug usage and etofenamate detection in post-race equine urine sample: A case report. *Biomed Chromatogr,* 37 (3): 1-6, 2022. DOI: 10.1002/bmc.5556
25. **Kabil E, Göktaş EF:** Development and validation of confirmatory quantitative and screening methods for 63 prohibited substances in horse urine using LC-MS/MS. *Chromatographia,* 88, 151-168, 2025. DOI: 10.1007/s10337-024-04384-2
26. **Kabil E, Zor TA, Göktaş EF, Güneş E, Yatanaslan L, Tektaş MH, İnceman B, Tufan M:** Detection of sildenafil and its 9 metabolites in a post-race horse urine sample: A case report. *J Pharm Biomed Anl,* 249:116343, 2024. DOI: 10.1016/j.jpba.2024.116343
27. **Göktaş EF, Kabil E, Yatanaslan L, Güneş E, Dirikolu L:** Simultaneous quantification of caffeine and its main metabolites by gas chromatography mass spectrometry in horse urine. *Biomed Chromatogr,* 36:e5445, 2022. DOI: 10.1002/bmc.5445
28. **Göktaş E, Kabil E:** Caffeine analysis in urine by gas chromatography mass spectrometry: A non-derivatization detection and confirmatory method. *İstanbul J Pharm,* 54, 233-239, 2024. DOI: 10.26650/IstanbulJPharm.2024.1349707
29. **Kabil E, Turhan E, Güneş E, Göktaş EF, Pekkaya S, Dirikolu L:** The analysis of arsenic and cobalt in horseracing in Turkey. **In,** *Proceedings of the 22nd International Conference of Racing Analysts and Veterinarians. Dubai*, UAE, pp 52-55, 2018
30. **Göktaş EF, Kabil E, Söylemez Yeşilçimen E, Dirikolu L:** Screening analysis of doping agents in horse urine and plasma with dilute and shoot using liquid chromatography high resolution mass spectrometry. *The Analyst,* 150, 773-792, 2025. DOI: 10.1039/D4AN01501K
31. **FDA:** Questions and answers on arsenic-based animal drugs. **In,** FDA. 2025. https://www.fda.gov/animal-veterinary/product-safety-information/arsenic-based-animal-drugs-and-poultry: *Accessed:* 10 Jan 2025.
32. **Popot M-A, Ho ENM, Stojiljkovic N, Bagilet F, Remy P, Maciejewski P, Loup B, Chan GHM, Hargrave S, Arthur RM, Russo C, White J, Hincks P, Pearce C, Ganio G, Zahra P, Batty D, Jarrett M, Brooks L, Prescott LA, Bailly-Chouriberry L, Bonnaire Y, Wan TSM:** Interlaboratory trial for the measurement of total cobalt in equine urine and plasma by ICP-MS. *Drug Test Anal,* 9, 1400-1406, 2017. DOI: 10.1002/dta.2191
33. **Chakraborti D, Mukherjee SC, Saha KC, Chowdhury UK, Rahman MM, Sengupta MK:** Arsenic toxicity from homeopathic treatment. *J Toxicol Clin Toxicol,* 41, 963-967, 2003. DOI: 10.1081/CLT-120026518
34. **Brewer K, Maylin GA, Fenger CK, Tobin T:** Cobalt use and regulation in horseracing: A review. *Comp Exerc Physiol,* 1,12, 1-10, 2016. DOI: 10.3920/CEP140008
35. **Kwak YB, Choi MS:** Identification of a metabolite for the detection of the hydrophilic drug diisopropylamine for doping control. *J Pharm Biomed Anal,* 234:115576, 2023. DOI: 10.1016/j.jpba.2023.115576
36. **Machnik M, Kaiser S, Koppe S, Kietzmann M, Schenk I, Düe M, Thevis M, Schänzer W, Toutain PL:** Control of methylxanthines in the competition horse: Pharmacokinetic/pharmacodynamic studies on caffeine, theobromine and theophylline for the assessment of irrelevant concentrations: Control of methylxanthines in the competition horse. *Drug Test Anal,* 9, 1372-1384, 2017. DOI: 10.1002/dta.2097
37. **Bennett RC, Steffey EP:** Use of opioids for pain and anesthetic management in horses. *Vet Clin North Am Equine Pract,* 18, 47-60, 2002. DOI: 10.1016/s0749-0739(02)00011-1
38. **EMA:** Committee for Veterinary Medicinal Products Atropine Summary Report. 1998. https://www.ema.europa.eu/en/documents/mrl-report/atropine-summary-report-committee-veterinary-medicinal-products_en.pdf; *Accessed:* 19 Dec 2024.
39. **Hazum E, Sabatka JJ, Chang KJ, Brent DA, Findlay JW, Cuatrecasas P:** Morphine in cow and human milk: Could dietary morphine constitute a ligand for specific morphine (μ) receptors? *Science,* 213, 1010-1012, 1981. DOI: 10.1126/science.6267691
40. **Hertzsch R, Emmerich IU, Lachenmeier DW, Sproll C, Monakhova YB, Aboling S, Bachmann U, Vervuert I:** Alimentary intake of opioid alkaloids by horses. Hazards due to poppy-containing feeds. *Tierärztl Prax Ausg G Großtiere Nutztiere,* 43, 35-43, 2015. DOI: 10.15653/TPG-140638
41. **Sams RA:** Review of possible sources of exposure of horses to natural products and environmental contaminants resulting in regulatory action. *Proceedings of the Annual Convention of the AAEP,* 220-223, 1997.
42. **Evered KT:** Traditional ecologies of the opium poppy and oral history in rural Turkey. *Geogr Rev,* 101, 164-182, 2011. DOI: 10.1111/j.1931-0846.2011.00085.x
43. **IFHA:** Detection Times on the Therapeutic Substances Controlled by International Screening Limits. 2025. https://www.ifhaonline.org/resources/DetectionTimes.pdf; *Accessed:* 30 Dec 2024.
44. **Koupai-Abyazani MR, Esaw B, Laviolette B:** Etodolac in equine urine and serum: Determination by high-performance liquid chromatography with ultraviolet detection, confirmation, and metabolite ıdentification by atmospheric pressure ıonization mass spectrometry. *J Anal Toxicol,* 23, 200-209, 1999. DOI: 10.1093/jat/23.3.200
45. **EMA:** Committee for Veterinary Medicinal Products Diprophylline Summary Report. 1998. https://www.ema.europa.eu/en/documents/mrl-report/diprophylline-summary-report-committee-veterinary-medicinal-products_en.pdf; *Accessed:* 15 Dec 2024.