An undergraduate forensic biochemistry laboratory experiment to detect doping in animal hair using LCMS

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Abstract

Doping using performance enhancing banned substances is a serious problem in almost every sport competition. Not surprisingly, the detection of these contra banned drugs is an area of active and continuous improvement and innovation by bioanalytical chemists. Additionally, most students working out in the gym and taking part in various sports need to be made aware of the doping and the health problems associated with it. Science or STEM students, in particular chemistry students, must not only be made aware of these issues, but also be taught that chemistry (and science) can provide solutions to such real-life issues. To this end, a newly developed forensic laboratory experiment is described that guides students to learn liquid chromatography mass spectrometry instrumentation (LC-MS) to detect four common doping drugs cortisol, dexamethasone, methyl prednisolone and flumethasone in camel hair samples.

In addition, the project is also designed to reinforce the importance of hair analysis as an additional sample matrix, complementary to saliva, blood and urine tests, in doping applications. In addition to learning various aspects of sample preparation, extraction, and LC-MS principles, students will also learn how to validate this method according to Food and Drugs Administration guidelines for intra and inter day precision and accuracy, recovery, stability and linearity.

This "applied forensic science" experiment was successfully implemented in a biochemistry undergraduate research course to enhance students' learning of doping issues as well as important bio-analytical and forensic biochemistry concepts. Student survey confirmed that this laboratory experiment was successful in achieving the objectives of raising awareness of doping control in students and illustrating the usefulness of chemistry in solving real-life problems. This experiment can be easily adopted in an advanced biochemistry laboratory course and taught as an inquiry-guided exercise. Such hands-on and engaging experiments should be part of undergraduate curriculum to foster deeper interest and innovation in STEM subjects to better prepare the next-generation workforce in science and technology.

Keywords: Forensic Science, Doping, LC-MS, bioanalytical experiment, hands-on training, undergraduate research

1. Introduction

Doping is a serious problem in almost every sport played around the world involving humans and animals [1, 2]. Doping enhances the performance of a person or animal and give it a competitive edge over other athletes. But these performance enhancement drugs could also seriously damage the health of an athlete or animal later in their lives, and therefore the use of these performance enhancers are strictly prohibited in different sporting competitions [3, 4].

Hence, in recent years, there has been a concerted increase in scientific activities focused on doping awareness and research, all aimed to clean our sports. It is well recognized that the general public, young university sportsmen and college students should all be made aware of the seriousness of the doping challenges we face in the recent times [5, 6]. In addition, this experimental design will also improve critical thinking and generic transferable skills among young chemistry graduates as they are often found lacking these skills [7-9].

Camel racing is one of the most popular sports in the Arabian gulf countries and Australia. The winning camels are rewarded with cash prizes worth millions of dirhams [10, 11]. These awards can sometimes motivate some of the camel owners to cheat in order to enhance their camel's speed; by doping the camels with different steroids like glucocorticoids. Glucocorticoids are a class of corticosteroids that are illicitly used in human and animal's sports owing to their anti-inflammatory and mood elevating as well as euphoric properties [12-14]. According to reports from camel research centres, exogenous administration of glucocorticoids is a common cause of ban from the camel racing for participants. Four main glucocorticoids usually found in camel are hydrocortisone, dexamethasone, flumethasone & methylprednisolone. Cortisol and methylprednisolone are endogenously produced in camel while dexamethasone and flumethasone are synthetically produced.

Camel blood or urine samples are currently being analysed in accredited laboratories across the world for determination of endogenous and synthetic glucocorticoids in camels for doping control [15-17]. But major disadvantages of urinalysis and blood analysis is that it only provides short term information for drugs consumption [18]. It can also show false positive for accidental single intake of drugs [19]. Hair analysis on the other hand provides a wider surveillance window of detection (month to year) and full history of drug use [20].Hair testing is very stable, easy to ship and store, non-invasive, tamper resistant and most convenient technique for control of drug doping. Hair test can complement blood and urine analysis [21].

Many other relatively simple chemistry experiments have been published in the past that can be incorporated in undergraduate chemistry curricula to raise awareness of doping in sports among chemistry students, and to show how chemistry can be used to solve practical, real-life problems of doping in sports. For example, our laboratory has previously published relatively simple chemistry experiments that show the applicability the analysis of nandrolone and stanozolol in university students gym athlete [21]. We have also conducted a study on primary outcome measures for new university students which were calculated

based on the portions of fruit and vegetables consumed, physical activity levels, units of alcohol consumed and smoking status at 6-month follow-up. Where it was concluded that targeted health behaviour of new university students should therefore focus on single health behaviours [22, 23]. In another study the use of fat burner Dinitrophenol was analysed [24] and many other studies involving hair analysis in university students are discussed [23, 25, 26]. Similarly, others have published educational experiments on the doping in sports [4, 27].

Multiple methods have been published for the determination of glucocorticoids in human hair [28, 29]. Many methods have described the analysis of glucocorticoids in camel saliva [30, 31], urine [32], blood [33, 34]and faeces [35]. Our literature review indicates that no method has been reported previously for the analysis of glucocorticoids in camel hair.

Although very promising, unfortunately there are no published reports of such approaches being taught to undergraduate chemistry and biochemistry students. There are, however, a few interesting educational articles on teaching chemistry/biochemistry students about doping and doping education [36-38]. This article also describes an experiment that we have developed and successfully used in an undergraduate biochemistry laboratory. In addition to teaching students how drugs can incorporate into hair along with how hair analysis could be used for detection of various glucocorticoids in camel hair. The described experiment also emphasized various factors that can effect detection of drugs in hair, which involve contaminants sticking to hair, hair colouring, hair growth cycle, segmental analysis of hair and hair length. There were four main objectives for this biochemistry laboratory:

1-Raise student awareness about the issue of doping in sports and their adverse health effects
2-Teach undergraduate students an advanced bio-analytical instrument that can be used to address doping related questions
3-To stress the importance of science and chemistry in solving real-life problems
4-To promote interest and innovation in STEM students by engaging them in an inquiry-based learning exercise

Additionally, the experiment is designed to be taught in a fashion that encourages inquiry-driven learning about the various parameters (stability, linearity, precision, accuracy etc. that can affect the hair analysis in doping application. Specifically, of the four levels of inquiry-guided learning in science (confirmation inquiry, structured inquiry, guided inquiry, and open inquiry) [38, 39], the described experiment would be very suitable for confirmation and structured inquiry approaches. These two approaches are usually employed with students who have not had previous experience with inquiry-guided learning.

For the “confirmation inquiry” style of teaching this exercise, students will be provided with the specific questions (e.g. how hair analysis can complement urinalysis and blood analysis) and a detailed procedure of how to do this. The instructor will also tell them the expected result and the students will essentially

confirm the expected results. This approach will reinforce students' skills in following specific laboratory instructions, collecting, recording, and analysing data. If this experiment is taught using "structured inquiry" style, students will be provided with specific questions (e.g. how do hair analysis can complement urinalysis and blood analysis) as well as detailed procedures. However, unlike the confirmation inquiry style, they will not be informed about the expected results; rather students will be asked to use the data to come up with possible explanations for their observations.

2. Experimental Procedures

1.1 Safety Requirements and Hazards

1.1.1 Standards and reagents

Dexamethasone, flumetasone, methylprednisolone, hydrocortisone and hydrocortisone-9,11,12,12-d4 were all purchased from Labco Ltd UAE. Dichloromethane, methanol, pentane, formic acid was purchased from Emirates Scientific Supplies Ltd, UAE along with LC-MS grade water and acetonitrile. Glass test tubes (15 ml and 6 ml), Glass Pasteur pipettes, HPLC vials with caps (2 ml) were also bought from Emirates Scientific Supplies Ltd, UAE.

As with any laboratory-based experiment, students should wear gloves, safety glasses, and laboratory coats during the course of the experiment. Formic acid is perhaps the biggest hazard in this laboratory. Extreme care should be taken while using this acid, especially avoiding any spills or respiratory inhalation. Solvents like pentane can cause skin cracking and proper care is necessary while handling this solvent. Glucocorticoids are colourless compounds and students should be very careful while pipetting. Proper care should be taken when using other reagents. The CAS numbers of all the chemicals used are listed below:

Methanol- CAS Number: 67-56-1
Acetonitrile- CAS Number: 75-05-8
Pentane- CAS Number: 109-66-0
Formic acid- CAS Number: 64-18-6
Dexamethasone- CAS Number: 50-02-2
Flumethasone- CAS Number: 2135-17-3
Methylprednisolone- CAS Number: 83-43-2
Cortisol- CAS Number: 50-23-7

It is strongly advised that the MSDS of these chemicals should be printed and made available to the students before the laboratory starts.

1.2 Pre-laboratory Lecture & Post-laboratory Discussion

At the beginning of the laboratory, students are first given a "generic lecture" on glucocorticoids (hydrocortisone was chosen as an example in this case), and the various techniques available to detect

doping in sports, including LC-MS based approaches. At the end of this pre-laboratory lecture, students are informed about the exact nature of the laboratory and how they will be divided into 4 individual students, each testing a specific parameter that can effect doping in hair analysis. At the end of the laboratory, results from each of the 4 students are compared and discussed (as described later) to promote students to realize for themselves the importance of various parameters for efficient hair analysis for doping applications.

1.3 Specimens

Camel hair samples were collected from different breeds of camels kept in a farm in Al Ain, UAE. Hair samples from three racing camels were also obtained from a racing camel owner. The hair samples were collected from the crest of neck (mane) area of the animal, cut directly at the skin surface. This area was chosen because it shows less variability in hair growth. The hair samples were stored individually in labelled, sealed plastic envelops. The study protocols were approved by UAEU research ethics committee. Blank hair was received from a healthy, non-racing camel in the same manner.

1.4 Sample extraction

Camel hair samples were washed with dichloromethane for few minutes to remove any contaminants and foreign objects sticking to hair samples. The hair samples were dried under air and normal temperature. The dried hair samples were pulverized using Mini-ball mill Pulverisette 23, Fritsch Germany. 20 mg of grinded hair was weighed and 50 µl of internal standard hydrocortisone-d4 (concentration 1 ng/ml) was added to all hair samples except blank matrix samples. 1 ml of methanol was added to hair samples and then samples were sonicated for 2 hours in an ultrasonic bath (Branson 5800, Danbury USA) at 40 $^{\circ}$ C. After ultra-sonication of samples for a minute, 1 ml pentane was added to the mixture. Samples were vortex mixed again for 2 minutes. Next the mixture was centrifuged at 1680xg for 10 minutes (Beckman TJ-6, Beckman UK). Few samples were re-centrifuged if clear extracts were not obtained. After centrifugation the top organic layer was separated into new Pyrex glass test tubes using Pasteur pipettes. The clear extract was evaporated using nitrogen gas sample concentrator at 40 $^{\circ}$ C (Techne, Bibby Scientific USA). The dried extract was reconstituted with 100 µl of methanol. 4 µl was injected onto the LC-MS/MS system.

1.5 LC-MS/MS Analysis

The LC-MS/MS system comprised of an ACQUITY ultra pressure liquid chromatography (UPLC) class-1 system (Waters, UK) and advanced tandem quadrupole mass spectrometer XEVO TQ-S (Waters, UK). The column compartment in ACQUITY UPLC system was maintained at 50 $^{\circ}$ C. An ACQUITY UPLC column BEH C18 (2.1 mm x 50 mm, 1.7 µm) was used for chromatography along with an ACQUITY UPLC BEH C18 VanGuard Pre-column guard, 130 Å, (2.1 mm X 5 mm,1.7 µm) for clean sample injections. Mobile phase flow rate was 0.5 ml/min. Mobile phase A consisted of 0.1% formic acid and 2 mM ammonium acetate in water, while mobile phase B consisted of 0.1% formic acid and 2 mM ammonium acetate in methanol. The gradient started with a 0.1 % mobile phase B, and changed to 99.9 % mobile phase B in 5 minutes, the gradient was kept for 4.9 minutes at this ratio. At 10 minutes the gradient

was equilibrated at initial ratio of 0.1 % mobile phase B and kept at this ratio for 1 minute. There was also a post time run of 2 minutes before the next injection to equilibrate the instrument.

Although the current exercise describes a single-timed assay, the same experiments can be carried out in continuous measurement mode. This modified experimental approach can allow students to calculate different concentrations of corticosteroids in camel hair to evaluate doping. Each of the 4 students carried out different part of the validation analysis like Intra–inter day precision and accuracy, Linearity, sensitivity and specificity, Extraction recovery and stability.

3. Validation

3.1 Calibration curve and analysis

The calibrants for analysis of hair samples were made by serial dilution of the top calibrant 2500 pg/mg in methanol. 20 mg camel hair was used The samples for the standard curve were processed as described in the materials and method section. The ratio of peak area of glucocorticoids to that of the internal standard was plotted versus the concentration of the glucocorticoids in the calibration standard and a least-squares linear regression analysis was performed. Values of unknown hair concentrations were determined from the regression line of this calibration curve. Quality controls were obtained by spiking corticosteroids into 20 mg of camel hair samples to produce concentrations of 156.2, 312, 625 and 1250 pg/mg. All solutions, calibrants and quality controls were stored at -20 °C with an expiry of 7 days, due to their short stability in methanol.

3.2 Student 1. Intra–inter day precision and accuracy

This student carried out the following experiments. The accuracy and precision of the assay was determined by analysing 20 mg camel hair samples spiked with four quality control (QC) samples of 156.2, 312, 625 and 1250 pg/mg of glucocorticoids. These QC samples were later assayed by LCMS/MS. To assess the inter-assay precision and accuracy, samples were analysed on five separate days. To assess the intra-assay precision, these same QC concentrations were analysed and compared during a single day.

3.3 Student 2. Linearity, sensitivity and specificity

This student carried out the following experiments. The ratios of glucocorticoids and internal standard responses were plotted by LC-MS/MS Quanlynx software (Quanlynx, an Application Manager included with Masslynx Software, is designed for quantitative analysis. Quanlynx automates data acquisition, processing and reporting) to determine the linearity. A calibration point was rejected as an outlier if the back-calculated concentration for a calibrator (on the basis of the corresponding calibration curve) deviated by more than 15 % at all concentrations covered by the calibration range, except at the lower limit of quantitation (LLOQ), where a deviation of 20 % was acceptable. A calibration curve was allowed with a minimum of four acceptable calibration levels.

The student calculated the specificity of the validated assay procedure by analysing 6 blank camel hair samples from camel not exposed to large concentrations of endogenous glucocorticoids. The analytical method was able to determine dexamethasone, flumethasone, methylprednisolone, hydrocortisone and hydrocortisone-9,11,12,12-d4 (internal standard) in camel hair without significant interference from other endogenous compounds.

3.4 Student 3. Extraction recovery

This student carried out the following experiment. The Absolute extraction recovery of glucocorticoids in camel hair were determined at three concentrations levels 312, 625 and 1250 pg/mg. The area ratio response of glucocorticoids to internal standard in the extracted sample divided by the area ratio response determined in an un-extracted sample and multiplied by 100 gave the percent recovery. These samples were extracted, as described earlier, except that the internal standard was added to the collected extract. The concentrations of the spiked camel hair samples were calculated from the curve and compared to the theoretical values in order to calculate the extraction recovery.

3.5 Student-4. Stability

Student 4 carried out the following experiment. The stability of camel hair was determined in processed sample extracts over at least 24 h period and also by three repeated freezing and thawing cycles. Stability of glucocorticoids in camel hair was tested at repeated freezing and thawing cycles.

Student prepared camel hair samples at concentration of QCL = 312 pg/mg, QCM = 625 pg/mg and QCH = 1250 pg/mg were subjected to three freezing and thawing cycles. The time span for freeze/thaw cycles was 72 h with each freeze/thaw cycle lasting for 24 h with time points 24, 48 and 72 h. The results obtained after each freezing and thawing cycle were expressed as a percentage change from the results for QCL = 312 pg/mg, QCM = 625 pg/mg and QCH = 1250 pg/mg in the intra-assay run. The test compound was considered to be stable if the percentage change from freshly prepared samples was within ±15 % of the nominally spiked level.

3. Results and Discussion

3.1 Camel hair analysis

All the four glucocorticoids were quantified in the camel hair samples along with a similar internal standard. 30 hair samples were obtained from a source farm in Al Ain. The owner disclosed giving medication to camels in the past year namely Terramycin and Trypanosoma vaccines. While 3 samples from racing camels were obtained from another camel owner in Al Ain. One of the racing camels were given corticosteroids for an injury.

Glucocorticoids (dexamethasone, flumethasone, methylprednisolone, hydrocortisone and hydrocortisone-9,11,12,12-d4 (internal standard)) were identified and quantified on the basis of their retention times and

the relative abundance of their respective product ions. The retention times, multiple reaction monitoring (MRM) transitions (showing precursor and product ions), respective molecular weights, cone voltages and collision energies are given in Table 1 below.

Table 1. Retention times, MRM transitions and conditions for glucocorticoids

Analyte	Retention Times	M. Wt. (M)	$[M-H]^-$ (-ve electrospray Ionisation)	Precursor - adduct $[M-H+HCOO^-]$	Fragments	Cone Voltage	Collision Energy
Hydrocortisone-9,11,12,12-d4 (Internal standard)	5.29	366.48	365.2	411.2	335.2	20	25
Hydrocortisone	5.09	362.47	361.2	407.2	331.2	20	20
					343.2		
					301.2		
Dexamethasone	5.82	392.46	392.2	437.2	361.2	20	20
					307.2		35
					325.2		
Flumethasone	5.67	410.45	409.2	455.3	305.2	20	35
					325.2		30
					379.2		20
Methylprednisolone	5.9	374.44	373.2	419.2	343.2	20	20
					309.2		35
					294.2		

3.2 Calibration curve and analysis

3.2.1 The following experiment was divided into 4 students as follows.

All the analytes, dexamethasone, flumethasone, methylprednisolone, hydrocortisone and hydrocortisone-9,11,12,12-d4 (internal standard) showed sharp, well-defined peaks at their respective retention times. Only two most sensitive MRM transitions (out of three as shown in Table 1 above) were chosen for the quantitative analysis of each glucocorticoid. The chromatogram shows internal standard on the top followed by flumethasone, dexamethasone, methylprednisolone, and hydrocortisone respectively.

3.2.2 Intra and Inter assay accuracy and precision (Student 1)

The inter-assay accuracy and precision were calculated from results obtained from quality control samples (N = 6) of hydrocortisone, dexamethasone, flumethasone and methylprednisolone analysed at four different concentration levels on three separate occasions (156.2, 312, 625 and 1250 pg/mg in hair samples representing LLOQ, QCL, QCM and QCH respectively), On the other hand, intra-assay accuracy and precision was calculated from QC's analysed on one occasion, see Table 2.

Table 2 Validation results showing intra and inter-day precision and accuracy and linearity

Analyte	QC's concentration	LOD/ LOQ	Linear range	r2	Intra-assay		Inter-assay	
	pg/mg	pg/mg			Precision, %CV	Accuracy, %	Precision, %CV	Accuracy, %
Hydrocortisone	312	0.15/78	78-2500	0.995	7.9	104.5	5.0	116.2
	625				3.4	98.5	2.9	105.5
	1250				4.7	100.6	1.1	97.4
Dexamethasone	312	0.15/0.30	19-2500	0.998	8.1	103.8	7.2	115.2
	625				2.6	97.8	3.5	107.5
	1250				4.2	95.2	5.3	101.3
Flumethasone	312	0.15/0.30	26-2500	0.998	8.6	96.8	7.5	118.4
	625				2.8	97.6	3.4	107.5
	1250				4.9	94.1	6.9	101.0
Methylprednisolone	312	0.15/0.50	0.5-2500	0.996	3.7	110.2	7.6	109.7
	625				4.1	111.8	3.3	102.9
	1250				7.0	104.1	3.9	101.0

LOD limit of detection, *% CV* coefficient of variation

3.23 Linearity, sensitivity and specificity (Student 2)

During the validation study, calibration curves were generated over a concentration range 0.15 to 2500 pg/mg. The method showed good sensitivity, specificity and linearity in the concentration range of 78, 19, 26, 0.5 to 2500 pg/mg for hydrocortisone, dexamethasone, flumethasone and methylprednisolone respectively. The curves were all linear with a mean coefficient of determination of 0.9998. The limit of quantitation was 78, 19, 26, 0.5 pg/mg for hydrocortisone, dexamethasone, flumethasone and methylprednisolone respectively. Using a signal-to noise ratio measure, the estimated limit of detection was 0.15 pg/mg for hydrocortisone, dexamethasone, flumethasone and methylprednisolone respectively. Furthermore, the percentage recovery of glucocorticoid samples, was well within the accepted limit of ±15 %, thereby showing no matrix effects as can be seen from the Table 3. No notable peaks were seen in the region of interest when six blank plasma samples were analysed. The retention time region of the chromatograph where glucocorticoid and internal standard was clear in these samples and demonstrated

the specificity of the validated analytical procedure. No interference from endogenous compounds or metabolites of glucocorticoids was found around the elution times, however few matrix peak was observed at a different retention time.

Table 3. Recovery of glucocorticoids when added to blank plasma showing no notable matrix effect (n = 6)

Concentration (pg/mg)	Recovery Mean ± SD (%)
156.2	98.75 ± 7.61
312.0	97.32 ± 4.22
625.0	99.20 ± 3.13
1250.0	98.59 ± 2.21

3.24 Recovery (Students 3)

We used 20 mg of camel hair incubated in 1 ml of methanol in an ultrasonic bath for 2 hours followed by liquid-liquid extraction using pentane produced a good recovery. Final recoveries were calculated during validation runs as shown in Table 2. The percent recovery of the three QC's is shown in Table 2.

3.25 Stability (Freezing and thawing cycles) (Student 4)

Three freeze/thaw cycles were performed at 24, 48 and 72 hours respectively. Quality controls at 3 different concentrations 312, 625 and 1250 pg/mg were analysed and compared for the 4 four glucocorticoids namely hydrocortisone, dexamethasone, flumethasone and methylprednisolone respectively. The samples were then extracted and injected. The mean concentrations of the stability samples were compared to the theoretical. The freeze/thaw cycle 1 shows quality controls analysed at time 0, then freeze/thaw cycle 2 shows quality control samples analysed at 24 and freeze/thaw cycle 3 at 48 hours respectively. The data indicated that the 4 glucocorticoids were stable in hair samples to at least three freezing and thawing cycles. The validation results indicated that the proposed method is more efficient in detecting the glucocorticoids, in camel hair even at very low levels when only ca. 20 mg hair was processed. Thus the, three freezing and thawing cycles showed that the 4 glucocorticoids were stable in camel hair. Many methods has been published for the determination of corticosteroids in human hair [40, 41], animal hair [19, 42-45] and currently there is no method reported for analysis of corticosteroids in camel hair.

3.26 Assay application

The hair analysis of 3 racing and 30 non-racing dromedary camels showed the following data for the four corticosteroids. Endogenous hydrocortisone was found in 13 camels in the range 30.7 to 935.35 pg/mg while in the rest hydrocortisone was lower than the limit of quantification. It was also interesting to note

that 1130.15 was the hydrocortisone found in one of the racing camels along with high concentrations of flumethasone 2575.5 pg/mg, methylprednisone 1155.8 and dexamethasone 29.3 pg/mg. Non-racing camels were also found with dexamethasone in the range 7.5-59.3 pg/mg, methylprednisolone in the range 5-66.25 pg/mg and flumethasone 0.7-1034 pg/mg. The concentrations of glucocorticoids found non-racing camel hair samples are shown in Table 4.

Table 4. shows the amount of glucocorticoids found in camel hair

Camel ID	Dexamethasone	Flumethasone	Hydrocortisone	Methyl prednisolone
	pg/mg	pg/mg	pg/mg	pg/mg
Camel 1	7.5	7.0	35.3	10.7
Camel 2	27.4	25.9	30.7	34.1
Camel 3	12.7	11.0	20.4	14.2
Camel 5	11.2	11.1	38.7	12.7
Camel 7	11.3	9.2	27.5	9
Camel 8	4.6	4.1	935.3	5
Camel 9	13948.9	14012.5	16406.1	13606.3
Camel 10	12.7	11.5	16.2	11.4
Camel 12	59.3	57.4	122.5	66.2
Camel 19	18.1	21.2	44.1	22.2
Camel 23	-	-	31.7	-
Camel 24	-	-	5.3	-
Camel 28	13.3	1034.8	-	-
Camel 29	29.3	2575.5	1130.1	1155.8
Camel 31	-	0.7	-	-

The newly developed and validated method could have far reaching impact in further studies of corticosteroids in camels. The proposed method might be applied to other human and animal hair samples as well in future studies for accurate quantitation of corticosteroids. This new method will also be instrumental in any future drug studies to control doping in racing camels.

3.27 Additional Suggested Points for Discussion (Post-laboratory)

Why is Hair analysis in doping application important? (All students)

Why hair analysis is preferred over blood and urine analysis? (Student 1)

How hair test is complementary technique to blood and urine analysis? (Student 2).

How the segmental analysis in hair important? (Student 3).

Lastly, what other glucocorticoids could be beneficial for doping test? (Student 4).

4. Student Survey

During the course of the laboratory sessions, it was obvious that the students remained engaged throughout the whole time, seemed to enjoy the experimental part, and participated actively in the data comparison and discussions at the end of the laboratory sessions. However, to gain a more quantitative assessment, an anonymous student survey was carried out using SurveyMonkey.com website. The result of the survey, as shown in Table 5, was very positive and seemed to indicate that the vast majority of the students enjoyed the laboratory session and found it a very useful experience. Furthermore, we were pleased to receive the feedback that the two main objectives of the laboratory were achieved: the students felt that after the laboratory they had a better awareness of issues related to doping in sports and a better appreciation of how chemistry can be applied to solve real life doping issues. As further evidence that such "applied" technology laboratories strongly resonate with chemistry students, when asked if they would like to see more such examples of how chemistry can solve real-life problems, all the students unanimously indicated "strongly agree or agree." Hence, we strongly believe that incorporating such laboratory experiments in chemistry curricula will be most beneficial to undergraduate chemistry students.

Table 5. Results from the student survey carried out anonymously after the laboratory session. Students' responses to a few selected questions (anonymous survey, n = 4).

	Statement	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
	N = 4 respondents					
1	I found this unit very useful	54%	42%	4%	0%	0%
2	The unit taught me new and valuable skills	23%	50%	27%	0%	0%
3	This unit made me more aware of issues related to doping issues	65%	23%	12%	0%	0%
4	I already knew most of the content presented	19%	23%	35%	23%	0%
5	I think this unit (this new information) will help me with my other classes	23%	58%	19%	0%	0%
6	The unit was too much work and took too much time	8%	4%	15%	42%	31%
7	I have a better appreciation of how Chemistry can be applied to "real life" doping problems	54%	42%	4%	0%	0%
8	It was not a very interesting or useful unit and was a waste of my time	0%	0%	0%	35%	65%
9	I enjoyed this unit and was glad that we took it in this class	69%	27%	0%	4%	0%
10	It will be nice to have more examples of how "chemistry can solve real-life problems"	85%	15%	0%	0%	

5. Conclusions

The newly developed and validated method for the determination of corticosteroids in camel hair is rapid (11 min), sensitive, specific, reproducible and robust, and very sensitive. It utilizes pentane a less toxic solvent for extraction. Due to the very small variability and high reproducibility this method has been proved to be suitable for use in further doping studies in animals especially camels, which also demonstrates the possible adequacy of this assay for clinical studies in animal's health and disease. The new hair test is a new ground breaking innovation to add in camel's blood and urine tests already in place for camel's health and disease. This new test will complement blood and urine test and provide a longer window of detection for corticosteroids in camel health and disease and doping control in camel racing around the world.

Graduating Chemistry students should not only be trained in the technical aspects of the discipline, but also be well-versed in current global issues of doping in sports and doping control. The experiment presented here offers a unique opportunity to expose chemistry students to critical doping problem and to showcase the applicability of chemistry to help solve real-life problems. In addition to raising awareness among students and highlighting applied biochemistry, various parameters that can directly affect hair analysis were studied and discussed. Furthermore, the inquiry-based and critical-thinking nature of the experiments ensures that students learn on their own the various validation parameters that can affect validation processes. Based on personal experience and student feedback, it is believed that such experiments can be an engaging and interactive way to achieve the aforementioned educational objectives.
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