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Isolation, Characterization and Quantification of Civetone from Civet Musk

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Abstract

Civet is an important commodity of commerce because of its use in perfume industry. It is also used to some extent in traditional medicine. Civetone is the main component of civet that is responsible for its characteristic odor. The amount of civetone in the musk of civet cat is not determined yet. In this project, rapid, simple and sensitive quantitative thin-layer chromatography method for the quantification of civetone in civet was developed and validated. The method involved extracting samples with dichloromethane by sonication, and analysis by CAMAG TLC scanner 3, after using a civetone dervatizing reagent. The densitometric scanning and analysis was done in absorbance mode at 371 nm. A new method for quantifying civetone in civet was developed in the course of this study. This involved taking a known amount of civet adding to it, a dervatizing agent 2,4-dintrophenylhydrazine, which now raises the absorption maximum to the visible range, thus making quantification work possible. The linear regression analysis data for calibration plots showed good linear relationship with r^2=0.999 in the concentration range of 1-5 µg $spot^{-1}$ with respect to peak area. The method was validated for accuracy, precision and recovery. The civetone value in crude civet samples, quantified by TLC was 0.8-1.2%.

Keywords: Civet; Civet musk; Civetone; Limu genet; Zibad

Introduction

Civet (Amharic: Zibad), one of the most well known natural products originating from Ethiopia, is a natural secretion of the civet cat (Amharic: *Tirin*) [1]. It has enjoyed considerable demand in commerce since ancient times because it is one of the basic ingredients for making perfumes. Although the civet cat occurs in several other African and Asian countries, Ethiopia has always been the leading supplier of civet, since over 90% of the world supply comes from this country [1]. The main aim of this article is to review the production of civet by the animal and assess current methods of monitoring quality of civet musk [2].

Civet cat (Civettictis civetta) belongs to a family of small carnivorous mammals called Viverridae or Mongoose family and is related to both hyena and cat [3]. The African civet has short and dense fur that is greyish in color, with black spot arranged in rows along their bodies [2-6]. Civet cat occurs in sub-Saharan Africa from 15° N to 24° S latitude. The east-west range extends from Senegal to Somalia [3,4]. In Ethiopia it is found over a wide area of the south west regions of Keffa, Wollega, Illibabor, Sidamo, and Shoa are the well-known (Figure 1).

The civet cat has a unique gland, located at the perineum, where a civet musk is produced and accumulated. The cat marks the area where it is present in order to attract its partner by pressing its gland against objects [4]. It's interesting to note that its unique scent, in particular when dilute, attracts human beings, who in the course of history have managed to use it as an important ingredient in the making of perfumes. Over the years, Ethiopians have managed to develop civet farms, in which civet cats are kept in captivity, given special diets to increase yield of the product. It was exported directly from Gondar and it was a major Ethiopian export in the 1800s. It is said that the Queen of Sheba (1013-982 BC) presented civet as a gift to King Solomon alongside ivory, gold or myrrh [2]. The civet musk is removed every 10 days from the gland using a spoon made from horn. On the average 300-400 g of civet musk can be harvested from each cat annually [1].

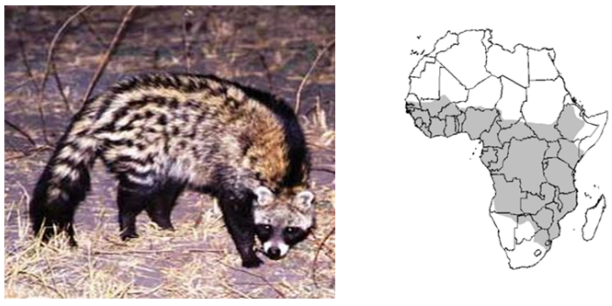

Figure 1: African civet cat and its distribution.

The Limu region of Keffa is one of the well-known centers of production and trade of civet till today. The regions of Sidamo, Shoa, Wollega, Keffa, and Illubabora still produce civet [2]. There are estimated to be more than 200 civet farmers in Ethiopia with about 4,000 civet cats in captivity at the time of this study. The civet cats are fed on a mixture of fruit and vegetables, maize meal and meat (1 kg/civet cat) or four eggs. The traditional unit of measurement for buying and selling civet is the *wecket* (also used for gold) where 1 *wecket* equals 42.4 g [1,7].

Civetone (9-cycloheptadecen-1-one, 1) is unsaturated 17-membered macrocyclic crystalline compound with a melting point of 32°C. It is isolated and characterized in 1926 important as ingredient of perfume [4]. Civetone was one of the first chemicals to be isolated from a mammalian scent gland [8]. In 1912 Sack isolated civetone from civet, and in 1926 Ruzicka elucidated its structure and other constituents. The characteristic strong, musky odor is due to this and an array of saturated and unsaturated cyclic compounds such as dihydrocivetone (2), dihydrocivetol (3) and (cis-6-methyltetrahydropyran-2-yl) acetic acid (4) [4]. Some of the isolated compounds from civet are listed below in Figure 2 [9,10].

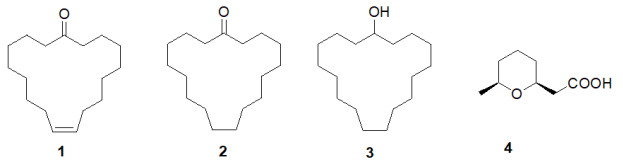

Figure 2: Constituents of civet musk.

Experimental Procedure

Civet samples, standards and chemicals

Analytical grade chloroform (99.98%), acetone (99.99%), petroleum ether, CH_2Cl_2, H_2SO_4, EtOH, and 2,4-dinitrophenylhydrazine (DNPH) , caffeine , $CDCl_3$, CCl_4, DMSO anisaldehyde reagent (anisaldehyde, H_2SO_4, EtOH and AcOH [0.5:0.5:9:0.1]) were used. Seven different civet samples were used in this study. One civet sample obtained from Ato Hamza Siraj, Jimma and the other six samples were obtained from Quality and Standard Authority of Ethiopia (QSAE) through W/r Genet.

Isolation of civetone

Three different methods were used in this work to isolate civetone from civet. First, 8 g of civet was taken and socked with $CHCl_3$ (80 ml) and sonicated for 20 min filtered and concentrated to give 6 g (75%) of $CHCl_3$ extract. All the $CHCl_3$ extract was packed on silica gel (70-230 mesh) and eluted using petroleum ether: $CHCl_3$. By increasing polarity, nine fractions were collected. Of these nine fractions, Fr-7 showed one yellow spot on TLC after spraying by anisaldehyde reagent and concentrated to give 120 mg mainly civetone (78% pure). Second, 1.3 g civet was taken and socked with $CHCl_3$ and sonicated for 20 min, filtered and concentrated to give 1.1 g (84%) of $CHCl_3$ extract. Acetone (10 ml) was added to $CHCl_3$ extract then sonicated for 4 min, filtered and washed to give 430 mg. This was applied on silica gel column (70-230 mesh) using petrol: $CHCl_3$ by increasing polarity, two fractions were collected. Fr-2 gave one yellow spot on TLC after spraying by anisaldehyde reagent and concentrated to give 13 mg (80% pure). Finally, 20 g of civet musk from one of the samples was taken, socked with CH_2Cl_2, then sonicated for 20 min, filtered and concentrated to give 17 g (85%). The CH_2Cl_2 extract was dissolved by $CHCl_3$ (5 ml) and applied on VLC by using VLC grade silica gel (100 g) and then eluted using petrol/$CHCl_3$ by increasing polarity, three fractions were collected. TLC of these three fractions was shown that the presence of civetone in Fr-2 and Fr-3. These two fractions were separately packed on column using the same solvent system as mentioned above and those fractions containing civetone were collected and weighed to be 146 mg and 280 mg respectively. The two were combined and washed by acetone; the acetone soluble and insoluble portions were separated and concentrated to get 200 mg (1% of civet sample) and 103 mg respectively. NMR shows that the acetone soluble portion was mainly civetone (86% pure). This was taken as a reference for analysis in this work.

Preparation of standards

For TLC analysis, two standards were prepared:

Standard solution I: To 75 mg of reference civetone in 25 ml volumetric flask $CHCl_3$ was added up to the mark to get 3 mg/ml or 3 µg/ µl Stock Solution.

Standard solution II: 1 ml of Solution I was diluted to 10 ml in a volumetric flask to give 0.3 mg/ml or 0.3 µg/ µl by $CHCl_3$. Both solutions were stored at 4c.

Preparation of Brady's reagent and civetonide

DNPH was dissolved in EtOH at elevated temperature, and filtered while it is still hot. The solution was allowed to cool; this cause crystallizes out. The crystal was separated from the solution and the melting point was measured (197-198°C) using Thiel tube. 30 mg of the recrystallized DNPH, 3-drops of H_2SO_4 and 2.5 ml of EtOH were mixed in 10 ml volumetric flask and $CHCl_3$ was added to get 3 mg/ml solution of DNPH. 1 ml of this solution was taken using 2 ml measuring pipette and then transferred into 10 ml volumetric flask and $CHCl_3$ was added up to the mark in order to get 0.3 mg/ml solution of a reagent known as Brady's reagent [11-14]. 20 g of civet sample was taken and socked with CH_2Cl_2, and sonicated for 20 min, filtered and concentrated. Brady's reagent was added to fraction one (Fr-1) allowed to stand for 10 min to react, then applied on column, and eluted by the same solvent system as mentioned above and five fractions were collected. Fr-3 and Fr-4 showed one yellow spot on TLC and the two were recombined and concentrated to give 300 mg civetonide.

Preparation of Civet samples for quantification analysis

1.5 g of each sample was taken and dissolved by CH_2Cl_2 (30 ml) and sonicated for 1 h, and then concentrated to give CH_2Cl_2 extract. To the CH_2Cl_2 extract, acetone (10 ml) was added, then filtered and concentrated. Two solutions were prepared for analysis:

Stock solution I: All acetone soluble portions transferred to 25 ml volumetric flak and filled by $CHCl_3$ up to the mark.

Stock solution II: 5 mg/ml solution of each sample was prepared by taking small amount and diluted farther.

Results and Discussion

Civet production in ethiopia

Civet production in Ethiopia is concentrated in the southern provinces of the country. The practice of keeping civet cat for the purpose of obtaining the civet musk is called civet farming. Since the cat is wild and ferocious, the farmer has to keep it in a secluded place using a trap for each cat. The trap has to be very secure so that the cat will not be able to escape. If it does it can cause severe bodily damage with its sharp teeth. Food is given to it while in the cage. To harvest the civet, it is taken out of the trap by holding its tail, and the civet is

removed from its gland using a special scooping tool made from horn and that looks like spatula as shown in Figure 3a. During the course of this project, researchers visited civet farmers in the Limu area of Jimma Province during June 2009 and found out that in Limu Genet Town there are still 5 families who farm civet. The indigenous knowledge of civet farming is restricted to some families flowing from generation to generation.

Figure 3a: A horn (A), a spoon (B) and civet cat inside a cage (C) (Photo by E. Dagne).

The decline is caused because of reduced demand for civet, which they believe is due to a provocative article that appeared in the National Geographic Magazine [15-17]. In that article one of the farmers in Limu, Aba Bulga Dina was interviewed by the Author of that article, who also published a picture of the farmer holding the tail of the civet cat while scooping civet from its gland. The author felt that this was tantamount to animal abuse. Such negative publicity must have caused European and North American customers of civet to shy away from importing Ethiopian civet. The other issue raised by the farmers that also contributed to the decline in civet demand is probably caused by greedy merchants and middle men who adulterate the civet product in order to gain more profit. They also pointed out that even the little product that they sale now, does not fetch enough return, because of the high cost of keeping civet cats. In order for the cat to produce good quality and quantity of civet, the farmer must feed it with meat and eggs.

In order for European customers to continue to buy civet, some measures must be taken at the production site. First and foremost, the handling of the civet has to be improved. One way of doing this is to use modern cages that are made for the purpose at the Bako Agricultural Station even though the price is not affordable to the farmers. Another significant measure is to reduce the middle men in the trade who probably are responsible for the adulteration. We have found that civet farming and trade in Limu is a highly guarded business, shrouded with mystery and secrecy. There are some indications that there is still interest in Europe for Ethiopian civet. However, to our surprise examination of the records at the Central Statistical Office and information obtained from the head of the information section confirms that there is no record since 1993 E.C. of quantity and value of civet export from Ethiopia. However, researchers are aware of the fact that the Quality and Standard Authority of Ethiopia (QSAE) certifies civet and facilitates its export [18].

Developing analytical methods that help distinguish good quality raw material from adulterated is one of the vital steps of this trade. Regrettably there is very little information in the literature on how to determine the level of civetone, which the most desirable active ingredient and marker of good quality civet. The probable reason for the lack of information on civetone level determination is because of the fact that this business is a monopoly of few perfume manufactures who probably have little inclination to publish their methods of analysis.

We have therefore made our main aim to develop a method for quantifying the level of the marker compound in order to be in a position to make judgment of the quality of civet. Our first undertaking was to isolate the marker compound in order to use it as reference substance in the analytical work.

Characterization civetone

Civet musk (20 g) was socked with CH_2Cl_2, then sonicated for 20 min, filtered and concentrated to give the crude extract (17 g, 85%). The crude extract was applied on Vacuum Liquid Chromatography (VLC) then eluted using petrol/ $CHCl_3$ of increasing polarity, three fractions were collected. TLC of two of fractions showed the presence of civetone. These two fractions were combined and packed on a column silica gel and was eluted with $CHCl_3$/petrol and those fractions containing civetone were combined to give 426 mg of impure civetone. This was washed with acetone and the acetone-soluble and acetone-insoluble portions were separated and concentrated to obtain pure civetone (200 mg). The NMR analysis of isolated civetone showed that, in the ^{1}HNMR spectrum the presence of triplet at δ 5.36 indicates olefinic hydrogen of civetone. The numbers of signals for hydrogen are few in civetone's because of symmetrical structure 1. shown in Figure 3b.

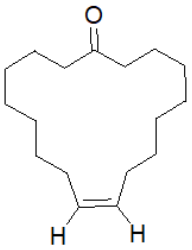

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Figure 3b: Symmetrical structure 1.

The ^{13}CNMR spectrum showed only 9 signals. This indicated the compound to be symmetrical. The signals at δ 212.4 and at δ 130.1indicated the carbonyl and olefinic carbons, respectively.

Quantitative analysis using ^{1}H-NMR

The civetone isolated and characterized above was used as a reference material in subsequent studies. Thus, the purity of the reference civetone was checked by quantitative NMR (qNMR) using caffeine as internal reference. As shown in Figure 4 the signal at δ 7.53 corresponding to the =C-H of caffiene was selected based on the following criteria: minimum overlap with other lines of the spectrum, minimum number of lines corresponding to spin-spin coupling, in order to obtain the best signal-to-noise ratio and precision, and absence of exchange phenomena. Integrals of the aromatic proton of caffeine and the olefinic protons of civetone were compared. This spectral region generally shows well-resolved NMR peaks displaying one-to-one correspondence between caffeine and civetone. The quantification by NMR spectroscopy needs to take into consideration the ratio of signals belonging to different compounds with respect to

those of an internal standard. This procedure can be carried out based on either peak integration (area) or peak intensity (height).

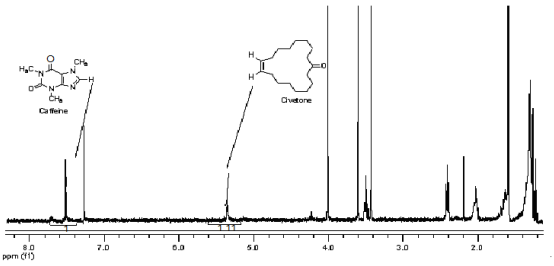


Figure 4: ^{1}H NMR of a sample containing caffeine standard (0.6 mg) and isolated civetone (1 mg) in $CDCl_3$.

In this work, integrations of selected signals were used to check the purity of isolated civetone. The integration ratio gives the mole ratio of civetone and caffeine in the NMR tube. The mass of caffeine is known, so the following formula can be used to calculate the mass of civetone in the NMR tube [19].

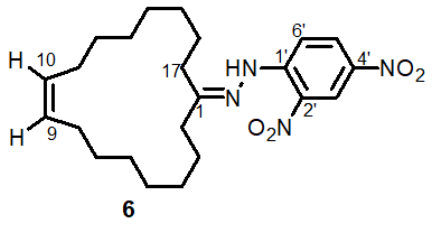
$$W_C = \frac{W_{ca} I_C MW_C}{I_{ca} MW_{ca}} \quad - - - - - - - - - - (Eq.1)$$

Where W_C is measured weight of civetone (in mg), W_{ca} is the weight of caffeine (in mg), MW_C the molecular weight of civetone, MWca the molecular weight of caffeine, I_C and I_{ca} are the integrals of selected resonances of civetone and caffeine, respectively. To check the purity of the isolated civetone 0.6 mg of caffeine and 1 mg of isolated civetone were added in to NMR tube the selected resonance are integrated as 1.11: 1 (civetone: caffeine) and the mass of civetone calculated as 0.86 mg which is 86% pure. The above method also can be used to also determine the of civetone present in the CH_2Cl_2 extract then extrapolate from this the level of civetone in civet.

Civetonide preparation

In this work determination of civetone level was also achieved through its derivatized form, using Brady's reagent as dervatizing reagent. In this paper the name civetonide is given for the dervatized form of civetone as the producer mentioned in section 3. Civetonide (6) obtained after column chromatography was recrystallized by dissolving it in EtOH at elevated temperature, filtering it while hot and allowing it to cool. The yellow crystal was separated from the solution using filter paper and its melting point was determined to be 58°C using Thiel tube. The sample has been sent abroad for MS analysis. The MS spectra showed the major peak at 429, 431, 430 for M-1, M+1 and molecular ion, respectively. This confirmed that isolated compound civetonide is pure.

NMR analysis shows all the characteristic signals of 6. The chemical shift of hydrogen on nitrogen of DNPH (Structure) was observed at δ 2.11 for primary and at δ 2.20 or secondary amines. After derivatization the proton of primary amine was not observed whereas the proton on secondary amine was observed at δ 11.23. This is due to the formation of double bond between carbon and nitrogen, and also the intermolecular hydrogen bond. The C-13 spectrum indicates 23 signals for each carbon, 17 for the civetone portion and 6 for DNPH portion of 6 because 6 is now unsymmetrical. HSQC showed that the two olefinic hydrogens at δ 5.29 on C-9 and C-10, but the two carbons shown at different chemical shift at δ 130.13(C-9) and δ 130.33(C-10) and the protons at δ 2.44 on C-2 and C-17 and also these two carbons have different chemical shifts at δ 38.57 and δ 30.92. The proton on nitrogen correlates with C-2', which is substituted with nitro group, is responsible for the formation of intermolecular hydrogen bond.



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Structure: DNPH

Thin-layer chromatographic analysis

Samples and standards were applied on pre-coated TLC plates as 6 mm bands, 14 mm distance between tracks using the 'spray on' technique. A CAMAG Linomat-5 (Switzerland) was used for sample application. TLC plates were developed with chloroform: pet ether (9:1 v/v) over a distance of 50 mm from the lower edge of the plate using CAMAG twin trough chamber (Switzerland), saturated for 10 min with the mobile phase by immersing filter paper. For pre-chromatographic dervatization method the developed plates were then dried in air for 5 min. Whereas for post-chromatographic derivatization method the developed plates were dipped in the CAMAG dipping chamber after filling the chamber about 50 mm from the bottom, immersed and taken out immediately, dried over CAMAG plate up to 110°C for 5 min. The spraying reagent was anisaldehyde.

The quantification of civetone was performed by densitometric evaluation in absorption mode at 371 nm using deuterium and tungsten lamps. A CAMAG TLC scanner-3 equipped with deuterium and tungsten lamps, set at 371 nm in absorption mode, was used for scanning the plates. The size of the scanning slit was adjusted to 5.0 × 0.45 mm and the scanning speed to 20 mm/s at a data resolution of 100 μm/step. The peak heights and areas of chromatograms were determined using winCATS software.

Samples preparation for TLC Analysis

Acetone was added to the CH_2Cl_2 extract of each sample and the soluble and insoluble portions were separated using filter paper. All the soluble and insoluble portions were transferred to 25 ml volumetric flask and filled with $CHCl_3$ to prepare Stock Solution I. Small amount of this solution of each sample was further diluted to 5 mg/ml to give Stock Solution II. Stock Solution II was used for both post-chromatographic and pre-chromatographic analysis.

Pre-chromatographic derivatization of civet samples

Determination of civetone level in civet sample can be done by using Brady's reagent as a pre-chromatographic dervatizing reagent. This method has not been used for this purpose and avoids the problem during quantification while using spraying or dipping techniques by giving clear chromatogram.

Equal volume of Brady's reagent and Stock Solution II of each civet sample were taken separately and allowed to react.

The standard solution was also derivatized before development by taking equal volume of Brady's reagent and Standard Solution II. 3.5.3. Development of the Optimum Mobile Phase.

The TLC procedure was optimized with a view to quantify civetone in different civet extracts. The mobile phase consisting of chloroform and petroleum ether (9:1 v/v) gave a good resolution, and a sharp and well-defined peak at R_f =0.77 for civetonide in the standard as well as in the extracts (Figures 5 and 6).

This enabled us to accurately quantify civetone in the diluted solutions.

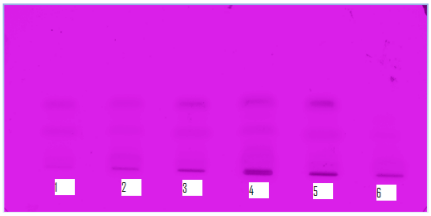

Figure 5: TLC of civetone standard and two extracts developed with chloroform petroleum ether (9:1). Left to right: Tracks 1, 3, 5, represent 1, 3, 5 µg civetone standards respectively; tracks 2 and 4 represent 2 and 4 µg civet extracts respectively; track 6 represents DNPH.

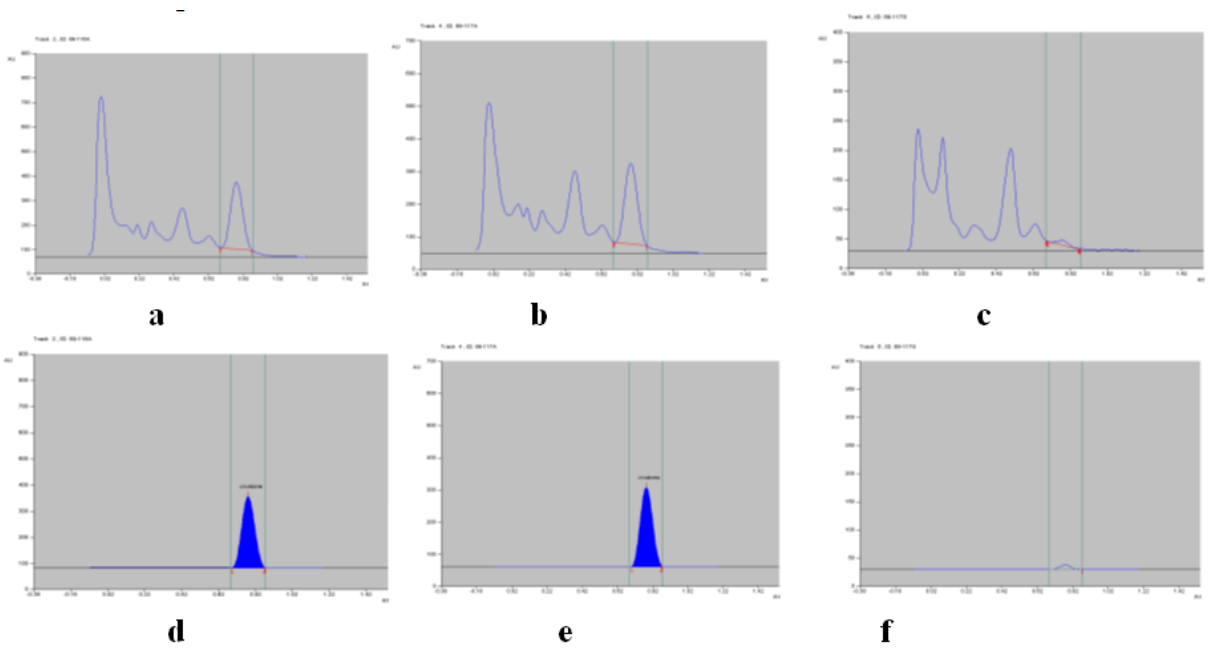

Figure 6: Detection of civetone in civet sample (b, e) and as reference (a, d) and DNPH (c, f) after chromatography with mobile phase.

Calibration curve preparation

From the standard solution II (0.3 µg/µL) 3.3, 6.7, 10, 13.3, 16.7 µL were spotted on the TLC plate to obtain concentrations of 1, 2, 3, 4, 5 µg $spot^{-1}$ of civetone, respectively. The data of peak areas were plotted against concentration to generate the calibration curve using winCATS software. The concentration of standards was selected in such a way that they could cover a wide range of concentrations detected in different samples. The developed TLC method for estimation of civetone showed a good correlation coefficient (r=0.99999) in the concentration range of 1 to 5 µg $spot^{-1}$ with respect to the peak height and peak area (Figure 7). Figure 8 displays the three dimensional image of the calibration curve of standards at 371 nm. No significant difference was observed in the slopes of standard curves.

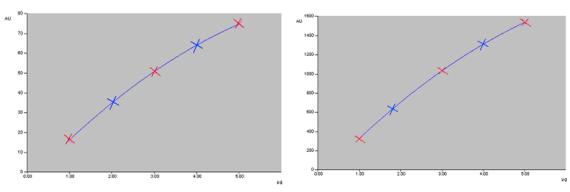

Figure 7: Calibration curves for civetone standards using polynomial regression modes: height (left) and area (right).

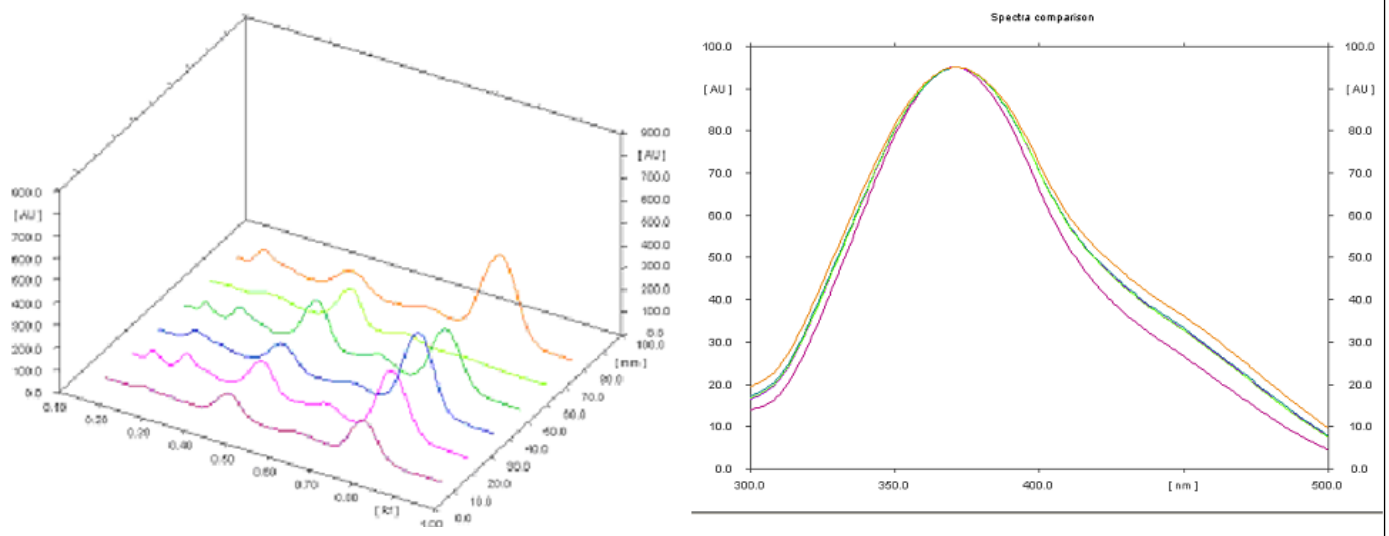

Figure 8: Three-dimensional image of the calibration spots for civetone at 371 nm, **b:** Spectral comparison of standard civetone and civet extract.

Method Validation

Precision

Repeatability of the sample application and measurement of peak areas were carried out using three replicates of the same spot (2.5 µg) of civetone and were expressed in terms of percent relative standard deviation (% R.S.D). The % R.S.D for repeatability of sample application (2.5 µg spot^{-1}), was found to be 2.4% as shown in Table 1.

Sample spot-1	Wt. applied	Area mean	Replicates				S.D	R. S .D	%R.S.D
			1	2	3	mean			
Civetone	2.5	-	2.332	2.224	2.292	2.283	0.0546	0.0239	2.4
Civetonide	4.3	2103	4.02	3.835	3.951	3.935	0.0935	0.0239	2.4

Table 1: Repeatability of the method (in µg).

The intra-day and inter-day variation for the determination of civetone was carried out at three concentration levels of 1.2, 1.3 and 1.4 µg spot^{-1}. The measurement of the peak areas at three different concentration levels showed low values of % R.S.D for intra- and inter-day variation, which suggested a very good precision of the method (Table 2).

Wt. of Civetone spot-1	Intra-Day				Inter-Day			
	Area mean	Amount mean	S.D	%RSD	Area mean	Wt. mean	S.D	%R.S.D
1.2	10560	1.165	0.0466	4.0	10410	1.057	0.0238	2.2
1.3	11421	1.271	0.0229	1.8	11612	1.282	0.0513	4.0
1.4	12640	1.365	0.0259	1.9	12670	1.341	0.0201	1.5

Table 2: Intra- and Inter-day precision of TLC method (n=3).

Recovery studies

Results of recovery study was shown in Table 3.

Amount sample	Spiked amount	Theoretical value	Experimental value	Recovery
1.2 µg	0.4 µg	1.6 µg	1.61 µg	100.6
1.2 µg	0.6 µg	1.8 µg	1.69 µg	94.0
1.2 µg	0.8 µg	2.0 µg	1.94 µg	97.0

Table 3: Results of recovery study.

The accuracy of the quantification was assessed in a recovery study. One of the samples was spiked with extra 0.4, 0.6 and 0.8 µg of the standard civetone and the mixtures were reanalyzed. This was done to check for the recovery efficiency of the method at different concentration levels in samples. The proposed method, when used for estimation of civetone, afforded recovery of 94% to 100%.

The peak purity of civetone was assessed by comparing the spectra at peak apex position of the spot. Good correlation (r=0.9999) was obtained between the standard and the sample overlain spectra of civetone (Figure 8).

Application to samples

Six samples of civet were analyzed by quantitative TLC method shown in Figure 9. Each of these samples analyzed three times using ordinary TLC and one times using HPTLC.

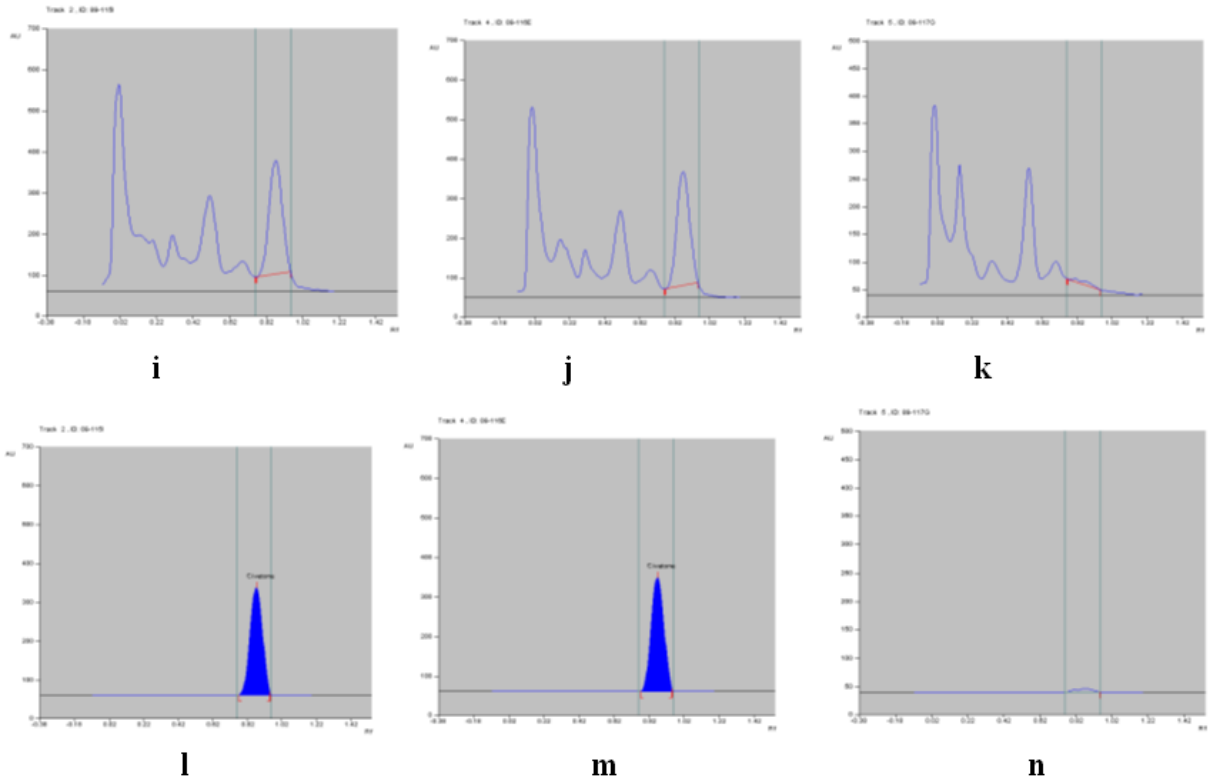

Figure 9: The spectral displays of sample A (i and l), sample B(j and m): control (k and n).

In the first run one sample was applied on one TLC, in the other rounds two samples were applied on one TLC. In this work no significant difference between ordinary and HPTLC on quantification.

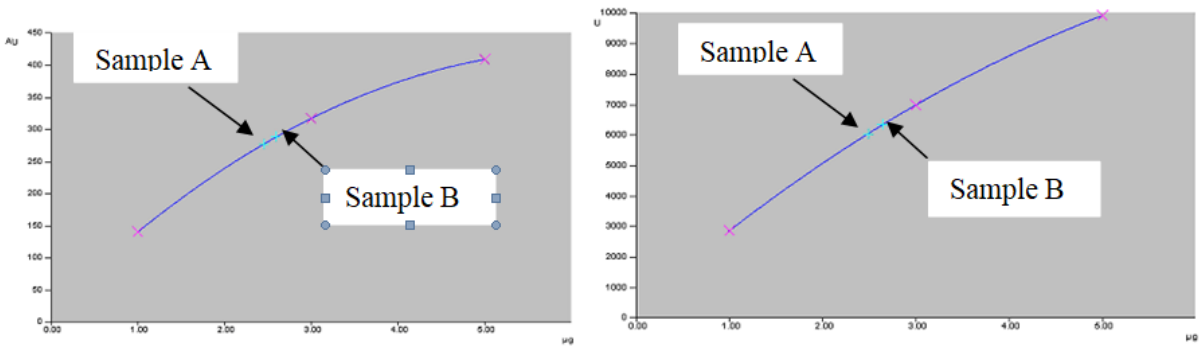

Figure 10: Quantification of civetone in two samples (A and B) on one TLC plate using polynomial (left) and linear (right) regression modes.

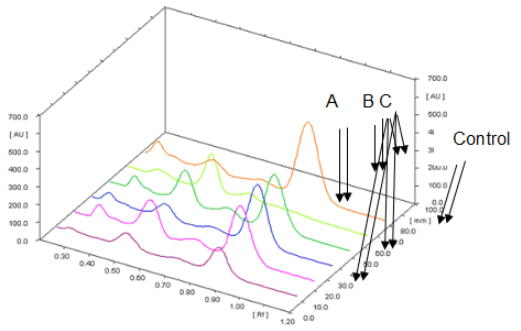

Figure 11: Three dimensional image of the calibration spots for samples (A, B) and standard (C) at 371 nm.

The analysis was done for acetone soluble and insoluble portions separately. The results are summarized in Table 3 and respectivy figure was shown in Figure 10. A single spot at R_f =0.77 was observed in the chromatogram of the civetone (in the form of civetonide) isolated from the extract along with other components. There was no interference in analysis from other components present in the extracts. These components appear in the chromatogram at significantly different R_f values as indicated in Figure 6.

The spectra display and the curves for estimation of civetone on TLC plate are indicated Figures 9 and 10, respectively. The three dimensional spectra of two samples and control also indicated (Figure 11). The amount of civetone in crude samples was around 1%, which means 1 g of civetone can be isolated from 100 g of crude civet sample. To confirm this one of the samples was analyzed in this work. 200 mg of civetone was isolated from 20 g of crude civet sample [20]. Results were shown in Table 4.

Civet	Amount spot-1 µg	% of civetone in acetone soluble				Mean	% Acetone insoluble	% Civetone in sample
		Ordinary TLC			HPTLC			
		1	2	3	4			
A	75	0.9	0.9	0.7	0.8	0.8	0.04	0.8
B	75	0.6	0.6	0.6	0.6	0.6	0.06	0.6
C	75	0.7	0.8	0.8	0.7	0.8	0.11	0.9
D	75	0.8	0.9	0.7	0.8	0.8	0.2	1
E	75	0.5	0.2	0.3	0.3	0.3	0.11	0.4
F	75	0.9	1.4	1.3	1.1	1.2	0.12	1.3

Table 4: Summary of qTLC results.

Conclusion

The developed quantitative thin-layer chromatography techniques are simple, rapid, accurate and precise methods for the determination of civetone in civet samples. The method showed appropriate recoveries and repeatability's and the proposed mobile phase effectively resolves civetone and thus, the method can be used for quantitative as well as qualitative analysis of civetone in different samples. The analysis results demonstrate that civetone can be effectively resolved and quantified in civet samples. For the revitalization of civet trade stakeholders of civet should share the problem of civet farmers by controlling the quality of civet, improve the handling of civet cat.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Guidelines

Ethics approval was not required for this research.

Conflicts of Interest

The authors hereby declare there is no conflict of interests.

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