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Research Article

IMPROVED ANALYTICAL METHOD FOR ESTIMATION OF ESSENTIAL OIL IN DRUG OINTMENT THROUGH RAPID STATIC CHROMATOGRAPHY HEADSPACE FOR QUALITY CONTROL ANALYSIS

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ABSTRACT

Objective: The objective for new methodology was to develop a rapid analytical method for drug quantification in ointment samples and eliminate the usage of hazardous solvents in the sample and standard preparation, less elution time of component of interest to sustained green chemistry applications.

Methods: Headspace (HS) chromatography was used along with gas chromatography (GC) having direct sample treatment with the help of calibration slope method.

Results: All essential oil (EO) was well separated from each other and eluted 1.6 times faster from traditional classical GC method. The present method does not require any hazardous solvents for sample preparation.

Conclusion: This method provides the accurate and precise results for EO added in ointment samples and can be used for routine quality control testing before releasing the final product release for the consumers.

Keywords: Essential oil, Gas chromatography, Headspace, Greener method and quality control.

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INTRODUCTION

Quality control testing in any pharmaceutical and health-care industry was always a challenging job to release the product in time by taking all special care during analytical testing, i.e., usage of toxic solvents, gases, and appropriate disposal for these experimental wastage [1]. Gas chromatography (GC) is frequently used for the estimates these oil contents used in the preparation of different products manufactured in pharmaceutical industries [2]. As essential oils (EOs) have medicinal value and used in the pharmaceutical industries for the manufacturing of various types of ointment, these oils are natural and having more impurities, which sometimes give erratic results during drug estimations and resulted out of specification (OOS). Due to these impurities, the method specificity was also challenges to get reliable results include complex sample preparation [3,4]. In current scenario, the EO estimation is the challenge in pharmaceutical Quality control lab due to complex sample preparation and usage of classical GC techniques, but no headspace (HS)-GC method is reported till date for these types of formulation. The major limitations for existing GC classical method have been described below as:

Complex sample preparation with the usage of chloroform as well as poor specificity in existing GC analytical methods lead to give OOS results during routine analysis. High elution time for a component of interest (about 45 min) for completion of single chromatography run deeply impacts the industry in terms of economic terms. The Traditional analytical method is not capable to separate unspecified impurity with the help of GC method. As it has been reported that there are many unspecified natural impurities, peaks of solvents used for sample preparation, and other ingredients peaks reported in chromatogram which cause coelution and erratic assay values.

To overcome all above discrepancy the innovative analytical method is optimized and partially validate in HS chromatography without the usage of any solvents for standard and sample preparation, i.e., direct samples explore to HS vial and inject to obtained precise and accurate results with shortest runtime to avoid diluents peak interference. Multilevel calibration curve is drawn to calculate each individual drug component. HS-GC is the most popular and rapid techniques for the analysis of major EO which are volatile in solid, liquid, and gas samples. In brief, the volatile components in the liquid or solid sample are transferred to a closed vial and kept to reach equilibrium between the sample and the vapor in the HS. A fraction of the HS vapor is sampled and introduced into a GC System. Injection of the analytes evaporated from the sample into the GC system could minimize the contamination of the instrument and deterioration of the GC capillary column [5-9].

In the present research work, estimation of EO is done by HS chromatography with direct injection of standards and test with no uses of preparation and usage of toxic solvents for sample preparation. The EOs used are oil of wintergreen (Methyl salicylate), Tarpin ka Tel (Pinene), Nilgiri Tel (*Eucalyptus*), and Pudinah ke Phool (Menthol) which is used in the manufacturing of pain relief ointment. The present research work is done to concern with the green chemistry to develop an analytical procedure and overcome the limitations of traditional methods causes exposure of solvents and gases to environment and safety of analytical chemist [10,11].

Tarpin ka Tel (pinene)

It is isomer mixture of pinene based molecule and having molecular formula $C_{10}H_{16}$ and molecular weight 136 g/mol. It is a transparent, colorless, or pale yellow liquid and has rosin irritating odor properties. It is soluble in alcohol, ether, benzene, chloroform, carbon disulfide, acetic acid, and organic solvents such as carbon tetrachloride; it has been used in varies the industrial application of organic synthesis, extraction fractionation turpentine by pinene, preparation of antiseptic ointment, synthetic borneol, and adhesives's original team [12].

Nilgiri Tel (eucalyptus)
Eucalyptol is a natural organic compound found in a colorless liquid. It is cyclic ether and a monoterpenoid. Many synonyms for eucalyptol is used for identification, namely, 1,8-cineol, 1,8-cineole, 1,8-epoxy-p-menthane, 1,8-oxido-p-menthane, eucalyptol, eucalyptol, 1,3,3-trimethyl-2-oxabicyclo[2,2,2]octane, cineol, cineole, and Nilgiri Tel as per Ayurvedic Pharmacopoeia. This drug is insoluble in water, miscible with ether, ethanol, and chloroform having molecular formula $C_{10}H_{18}O$ with molecular weight 154.249 g/mol. Drug is used in a cream, ointment, and lotion for prepared for pain relief and for speeding up the healing of wounds and ulcers [13].

Oil of wintergreen (methyl salicylate)
Methyl salicylate (oil of wintergreen or wintergreen oil) is an organic ester naturally produced by many species of plants, particularly wintergreens. Oil of wintergreen is miscible in diethyl ether, ethanol, glacial acetic acid, and soluble in chloroform having molecular formula $C_8H_8O_3$ with molecular weight 152.1494 g/mol. The drug is used for temporary relief of minor aches and pains caused by arthritis, simple backache, strains, sprains, and bruises. Methyl salicylate/menthol cream is a topical analgesic [14].

Pudinah ka Phool (Menthol)
Menthol is an organic compound and can be prepared by synthetically or obtained from corn mint, peppermint, or other mint oils. It is a waxy, crystalline substance, clear, or white in color having slightly solubility in water. It is used mostly in pain relief, anti-inflammatory ointments. Menthol is volatile in nature, having formula $C_{10}H_{20}O$ with molecular weight 156.27 g/mol. [15].

MATERIALS AND METHODS

Reagent and Solvents
No solvents used for standard and test preparation, i.e., direct weighing for component of interest and injection for mixtures of standards and test separately to HS-GC. The reference materials are used from (Indian Pharmacopoeia, 2017) IP their purity used 99.0% for reference standard. Perkin Elmer's GC, Elite-624, 30 m (Length) × 0.53 mm (id), 3 µ (thickness) capillary column were used throughout the experiment.

Equipment
Throughout the measurements and quantifications, Perkin Elmer's Clarus 580 with turbo 40 HS sampler was used with Empower software from Waters Ltd., MILFORD, MA 01757 USA was employed.

Chromatographic conditions
The EO samples were analyzed by HS-GC - 40 0.25 µm. Helium was used as a carrier gas. The conditions used for HS-GC are given in Tables 1 and 2, respectively.

Standard and test preparation
Standards calibration curve
Density of standard solutions weighing in g/ml and observed for Tarpin ka tel (Pinene), Nilgiri katel Eucalyptol Pudinah ke phool (Menthol), and Oil of wintergreen (methyl salicylate) is 0.86 g/ml, 0.900 g/ml, and 0.89 g/m land 1.174 g/ml, respectively.

1 ml of each standard was taken into HS vial (for solid or wax, weight equivalent to 1ml based on density in g/ml) and was mixed by shaking to prepare a standard solution.

10 mg of microcrystalline wax (ingredients used in the ointment) was taken into HS vial and spike 1 µl of above standard into it and crimped and treated this as standard, which was used for calculation of test concentration as given below:

$$\text{Amount in mg of each analyte in 1 µl} = \frac{\text{Density of standard component}\left(\frac{g}{ml}\right)}{\text{No of components in mixture}} \times \frac{1}{1000}$$

Table 1: GC conditions

Parameters	Conditions
Instrument	Perkin Elmer Clarus 580 GC
Column	Elite-624, 30 m×0.53 mm, 3 µm
Injector	Capillary split-split less injector (CAP)
Liner	4 mm liner filled with Silanized wool
Injector temperature	240°C
Carrier gas and flow rate	Helium@3 mL/min
Split (ratio)	30:1
Oven program	80°C–1.0 min 12.5°C/min. 200°C–2.0 min Runtime: 12.60 min
Detector	FID (range 20, Attenuation -3)
Sample injection diluent	HS no diluent is used (elimination of $CHCl_3$ which is used as diluents for the sample preparation)

HS: Headspace, GC: Gas chromatography, FID: Flame ionization detector

Table 2: HS chromatography conditions

Parameters	Conditions
Instrument	Perkin Elmer Turbo Matrix 40
Vial temperature	160°C
Needle temperature	170°C
Transfer line temperature	180°C
GC cycle time	20.00 min
Vial equilibration time	30.00 min
Pressurization time	2.0 min
Injection time	0.05 min
Withdrawal time column pressure (PPC)	0.2 min 15 Psi

HS: Headspace, GC: Gas chromatography

Test preparation: 10 mg of sample was taken into HS vial to crimp and run on HS-GC and flame ionization detector. In 1 µl standard mix number of milligram of solvent present was calculated and response of each component in sample chromatogram was observed which was correlated with standard response to get the amount of each component present in 10 mg of sample and multiply with 10 to get % of each in 100 mg of sample (remove the area from Microcrystalline Wax from sample) as given below:

$$\text{Percentage}(\%)\text{ of each analyte in sample} = \frac{\text{Area in sample} \times \text{Amount in standard}}{\text{Area in standard}} \times 10$$

Method validation approach
System precision and recovery studies have been conducted for HS-GC method.

Protocol for calibration plot/linearity curve
The standard stock solution was prepared same as per standard preparation procedure described in standard calibration curve preparation by mixing 1ml or equivalent to 1ml of each standard.

1 µl, 2 µl, 3 µl, 4 µl, and 5 µl of above standard solution was taken into five separate HS vials and crimped immediately to run on HS-GC to plot calibration/linearity curve.

10 mg of the sample was weighed into HS vial which further crimped and runs it on HS-GC. This sample were processed for chromatogram against calibration plot to know concentration in the sample.

Correlation coefficient was calculated for each component of interest. Results were evaluated as per Table 3.

Sample recovery study

10 mg of Microcrystalline Wax was weighed and put into HS vial which further crimped, this is taken as Blank.

10 mg of Microcrystalline Wax was weighed another and put into HS vial and spiked with 80%, 100%, and 120 target % of standard, which further crimp and inject in HS-GC for evaluation of relative error %, which should be 2% relative standard deviation (RSD) for average response recorded in each concentration injected.

Microcrystalline Wax represents sample matrix and if any Carryover/ Contamination was observed in M_{wax} Blank. It should be removed or deducted from spiked one. Results are evaluated as per Table 4.

System precision

Injected 3 standard Concentration and measure the area response for Relative standard deviations % described in Table 5.

RESULTS AND DISCUSSION

Comparison between classic GC experiments HS–GC for essential oil estimations

GC is a traditional method frequently used in the pharmaceutical labs for drug and molecular identification and quantification. Fig. 1a represents GC chromatogram where the run time was 25 min, and elution of each essential oil is at minimum 12.56 and for the maximum was 19.25 min which is the internal standard peak, which is not used in HS-GC due to precise and accurate reproducibility as compare to GC analysis. The same studies are run on HS and the elution of oil at minimum retention time - 8.43 and maximum at 11.46 and end with 15 minutes runtime which shows significant improvement in overall 1.6-time reduction in experimental time showed in Fig. 1b for chromatogram correspond to HS. Table 6 represents overall return on investment (ROI) of HS-GC in comparison with GC.

Traditional method required complex preparation procedure using chloroform ($CHCl_3$), the present study does not require any solvents for standard and sample preparation. Only direct sampling is exploited in HS vial and treated as per HS conditions described above. By considering the Green analytical approach, solvent elimination is focused and achieved as per ROI document for overall evaluation.

Method validation outcome

Specificity

Fig. 2 demonstrates the specificity of the method by identification of all essential oil, which was separated from each other having no interference due to blank (Wax) and placebo.

Linearity of response

The linearity of essential oil has been accessed at the different concentration by injecting 1 µl, 2 µl, 3 µl, 4 µl, and 5 µl range shown in Fig. 3, which was depicted by linear regression analysis revealed correlation coefficients, $r^2;>r^2=0.99$.

Accuracy

Recovery for essential oil was checked at different concentration (80%,100%, and 120%) found within the specification limit which is 98% to 102% within <2 % RSD

Precision

System was checked and found in within acceptable RSD <5%. Results were shown in Table 5 and overlay of chromatogram described in Fig. 4.

CONCLUSIONS

On the basis of this study, it appears that the use of this currently developed HS method for the quantification essential oil (EO) is much faster and robust method as compare to classical GC analysis in product formulation area is practical. The time reduction and solvent elimination characteristics of current HS method are very advantageous, compared

Table 3: Linearity curve evaluation

Analyte a name	Density (g/ml)	Amount of each analyte in standard (mg)					R^2 value
		1 µl	2 µl	3 µl	4 µl	5 µl	
Tarpin ka Tel (Pinene)	0.86	0.215	0.43	0.645	0.86	1.075	0.9975
Nilgiri ke Tel (eucalyptol)	0.9	0.225	0.45	0.675	0.9	1.125	0.9997
Pudinah ke Phool (menthol)	0.89	0.2225	0.445	0.6675	0.89	1.1125	0.9989
Oil of wintergreen (methyl salicylate)	1.174	0.2935	0.587	0.8805	1.174	1.4675	0.9982

Table 4: Recovery study - represent the recovery of individual essential oils

Concentrations %	Area response	True area theoretical	% RE
80	66235.234	67527.928	98.09
100	83706.34	84409.91	99.17
120	99823.624	101291.892	98.55
Tarpin oil		Avg	98.60
		SD	0.54
		RSD	0.55
80	50298.345	52867.272	95.14
100	64387.12	66084.09	97.43
120	78329.366	79300.908	98.77
Nilgiri oil		Avg	97.12
		SD	1.84
		RSD	1.89
80	59893.76	60931.44	98.30
100	76054.34	76164.3	99.86
120	91265.208	91397.16	99.86
Pudina ke phool		Avg	99.34
		SD	0.90
		RSD	0.91

RE: Relative error, RSD: Relative standard deviation, SD: Standard deviation

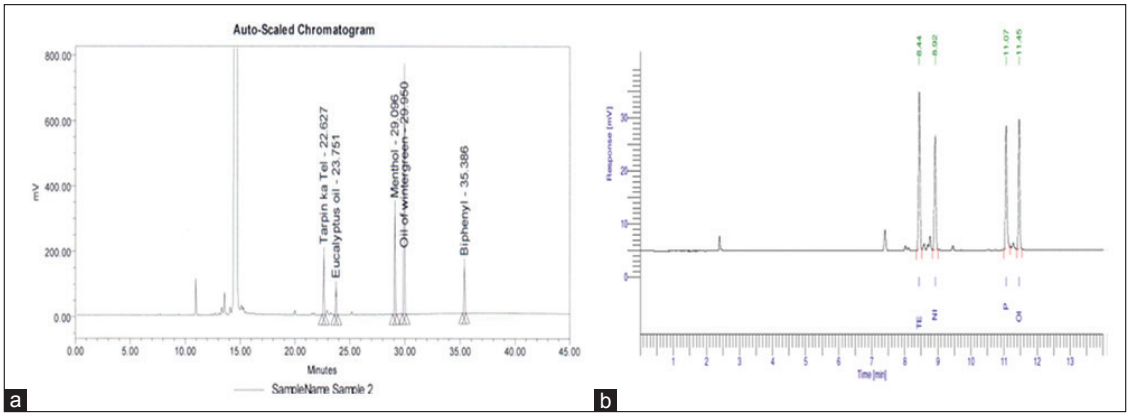

Fig. 1: Gas chromatography (GC) chromatogram of all essential oil by GC-runtime 45 min (a) and by headspace runtime 12 min (b)

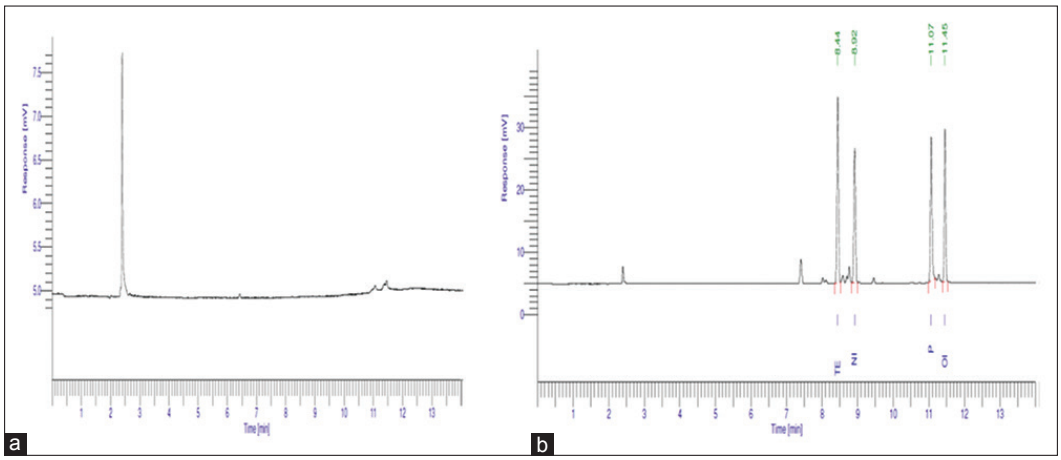

Fig. 2: Sample chromatogram of blank (Wax) (a) and with spiked standard in Wax (b) for specificity

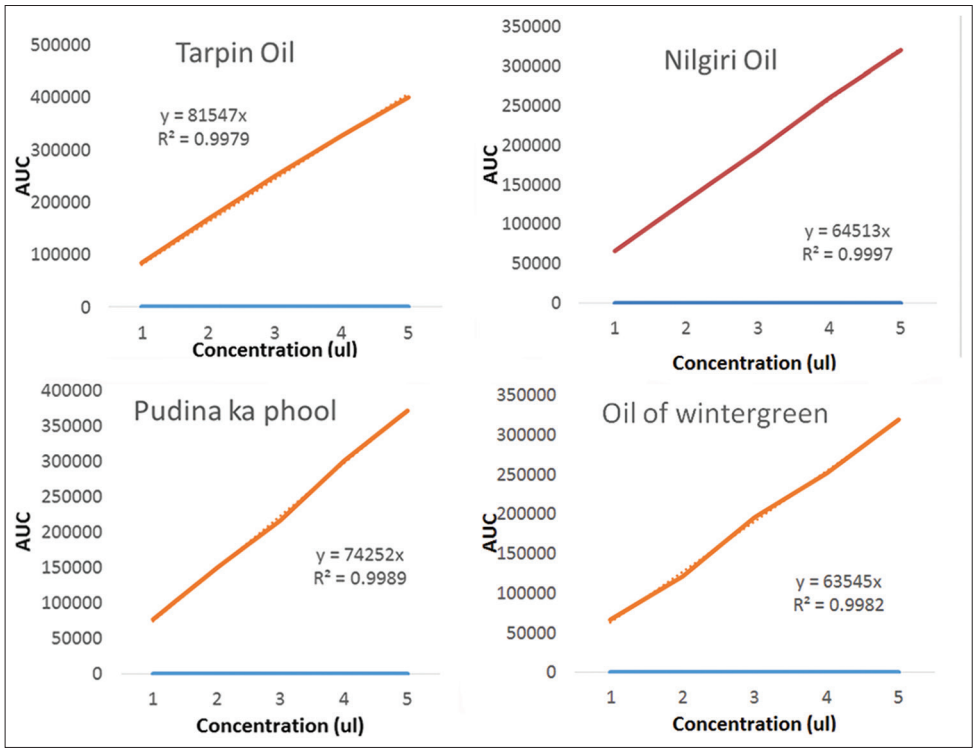

Fig. 3: Showed linearity optimization for all essential oil from 1ul to 5 µl concentration

Table 5: System precision

Inj	Terpin ka tel		Nilgiri ke tel		Pudinah ke phool		Oil of wintergreen	
	Time (min)	Area (µV.s)	Time (min)	Area (µV.s)	Time (min)	Area (µV.s)	Time (min)	Area (µV.s)
STD Inj 1	8.44	88077.95	8.92	70555.96	11.07	70157.04	11.46	66622.77
STD Inj 2	8.44	87135.64	8.91	66000.01	11.06	71461.64	11.45	62300.97
STD Inj 3	8.44	84409.91	8.92	66084.09	11.07	76164.30	11.46	65985.39
Avg	8.44	86541.17	8.92	67546.69	11.07	72594.32	11.46	64969.71
% RSD	0.04	2.20	0.04	3.86	0.04	4.35	0.04	3.59

RSD: Relative standard deviation

Table 6: Return on investment using HS-GC

Elements	Traditional GC method	HS-GC	Comments
Runtime	25 min/injection	15 min/injection	In current HS method, runtime is~1.6 times shorter
Sample preparation time	60 min	10 min	No sample preparation. Direct weigh the samples in vial and inject
Solvent requirement	300 ml	No solvents usage	Elimination of chloroform
Cost on testing/sample	750 INR	150 INR/sample	5 times cost effective

HS-GC: Head space-gas chromatography

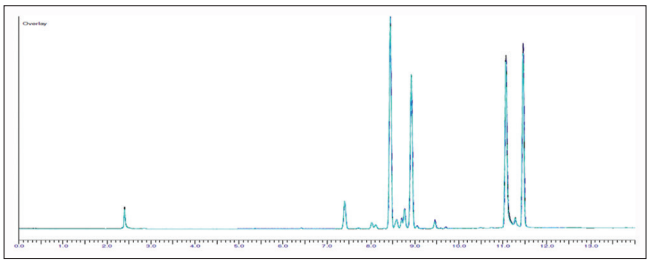

Fig. 4: Overlay chromatogram for system precision

to the most widely used conventional GC technique. Shorter runtime HS-GC method was developed with proper resolution between all peaks.

Direct sample analysis without dilution used for HS sampling. Quantification was done using standard spike method and calculating the response of sample against a standard with the help of density of components as shown in the calculations. Precision was performed for three injections and % RSD observed not more than 5.

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