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Original Article

Fingerprint of Tiger Balm® By Thermal Desorption Gas Chromatography Mass Spectrometry

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ABSTRACT

Introduction: Tiger Balm® is a blend of volatile oils used for many years in control of muscle cramps and headache. **Objectives:** To establish a quick and accurate method of analysis for Tiger Balm®. **Materials and Methods:** GC-MS and TD GC-MS were used in parallel to define the metabolites in Tiger Balm® blend in comparison with high quality standards in confirmation of the metabolite identities. **Results:** TD GC-MS was more efficient in showing the 1:1 relative abundance of camphor and menthol which can be taken as a chemical marker of this herbal medicine. **Conclusion:** In this work we applied efficiently the use of TD GC-MS in quality analysis of semisolid herbal medicine with volatile scents without the need of tedious pre-treatment with organic solvents, which is required by using GC-MS.

Key words: Tiger Balm®, TD GC-MS, GC-MS, Camphor, Menthol.

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INTRODUCTION

The medicinal agents' family is expanded continuously and creating a strong motive for developing and using sophisticated analytical methods for their evaluation.[1]

Nowadays modern chromatographic techniques use chemical markers, which may not be therapeutically active, to establish the standardization for herbal products. In many phytomedicines the bioactive constituents are not known, so in this case the products may be standardized depending on the content of certain marker compounds which are ranked as their chemical characteristics.[2] Phytomedicines with aromatic components also find many applications for either symptomatic relief or even cure of many diseases. Tiger Balm® (TB) is a mixture of camphor, menthol, cajuput oil, mint oil, cassia oil and clove oil which has rubefacient and analgesic properties and provides relief from the pain associated with the muscular aches, arthritis, and rheumatism. Gas chromatography (GC) is a very efficient analytical technique for detection and quantification of volatile compounds. When it is coupled to mass spectrometry (GC-MS), structural information can be obtained for their identification. When sorbent tubes/traps are used for sampling of volatile compounds, they can be anylysed in combination with GC-MS after thermal desorption (TD GC-MS).[3] The aim of this study is to apply TD-GC-MS as a fast and accurate method for detection of aromatic entities and control of the quality of phytomedicines with volatile compounds. TD GC-MS has an advantage to pass the tedious ordinary methods which involve extraction and/or preparation of derivatives resulting in the delay of reports revealing.

MATERIALS AND METHODS

Standards and herbal ointment

α-pinene, β-pinene, α-phellandrene, β-phellandrene, o-cymene, Racemic-menthol, L-menthol, L-menthone, eucalyptol, caryophyllene, α-terpineol, eugenol, limonene, camphene, γ-terpinene and n-alkanes C_8-C_{20} were supplied from Sigma-Aldrich with purity exceeding 96%. TB (Red) was purchased from Boots Pharmacy (UK).

GC-MS (method A)

Agilent 7890A gas chromatography systems, coupled to an Agilent MS model 5975C MSD with triple axis detector (Agilent Technologies, USA) used to check TB samples. 20 mg of TB was dissolved in hexane and 1 µl of the solution was injected directly into the GC-MS injection port. The GC proceeded in an oven with temperature program: initial 60°C for 0 min, raised to 150°C at 5°C/min, then raised to 300°C at 10°C/min, and held at 300°C for 2 min under a constant pressure (10 psi). The MS was fitted with electron impact (EI) source at 70 eV with an ion source temperature at 230°C. Mass spectra data were acquired in the scan mode in m/z range 16-600. The volatile compounds were identified by matching their EI-MS spectra with those in the NIST 2011 Mass Spectral Library using MSD Chemstation (Agilent Technologies, USA). They were further confirmed by comparing with authentic standards and/or their retention indices (RI), which were calculated using alkanes C_8-C_{20} standard, assayed under the same conditions, with literature data where the RI measured on the same capillary column. The percentage relative amount of individual volatile compounds was expressed as percent peak which is relative to total peak areas (n=3).

TD GC-MS (method B)

The TD GC-MS system consisted of UNITY-2 thermal desorption unit (MARKES International Ltd, UK) fitted to the previously mentioned GC system, preparation of sample was simple and direct, 100 mg of TB were put in a 20 ml Chromacol EPA vial (Fisher Scientific, UK) previously flushed with nitrogen gas. The vials were then sealed with white silicone/PTFE seal, and left for 30 min for saturation purposes of the head space, 20 mL of the headspace air were taken through a needle and a stainless steel desorption tube (C2-AA XX-5032 tube, Tenax TA/Carbograph TD, MARKES International Ltd, UK) by pulling a tightly sealed syringe with a flow of 20 ml/min. The tubes were subsequently placed in the UNITY-2 thermal desorption unit and pre-purged for 1 min for split flow of 20 ml/min. The volatile compounds were desorbed at 200°C for 3 min at a flow of 60 ml/min helium that passed the cold trap (graphitised carbon, U-T11GPC-2S, MARKES International Ltd, UK) and held at -15°C with no inlet split. After pre-trap fire purging for 1 min, graphitised carbon

Table 1: Volatile compounds identified and quantified by the direct injection of TIGER BALM® into the GC-MS system

no	RT (min)	Compound name	RI	% ± SD (n=3)	
1	5.054	alpha-Pinene	927	0.35	± 0.07
2	5.311	Camphene	942	0.03	± 0.01
3	5.735	beta-Phellandrene	966	0.13	± 0.01
4	5.791	beta-Pinene	970	0.19	± 0.02
5	6.494	(+)-4-Carene	1010	0.14	± 0.02
6	6.630	o-Cymene	1018	0.08	± 0.01
7	6.680	D-Limonene	1021	0.09	± 0.008
8	6.729	Eucalyptol	1024	10.77	± 0.50
9	7.220	gamma-Terpinene	1053	0.26	± 0.03
10	8.727	Camphor	1142	27.59	± 1.11
11	8.850	Trans-5-methyl-2-(1-methylethyl)-Cyclohexanone (Trans-Menthone)	1150	2.92	± 0.28
12	9.028	Cis-5-methyl-2-(1-methylethyl)-Cyclohexanone (Cis-Menthone)	1160	1.11	± 0.19
13	9.184	Menthol	1170	36.26	± 0.99
14	9.497	alpha-Terpineol	1189	4.29	± 0.1
15	10.356	Pulegone	1244	0.28	± 0.002
16	10.680	3-methyl-6-(1-methylethyl)- 2-Cyclohexen-1-one	1265	0.08	± 0.005
17	11.015	[1S-(1α, 3β, 6α)]-3,7,7-trimethyl-Bicyclo[4.1.0]heptane	1287	2.85	± 0.23
18	12.086	Eugenol	1360	9.13	± 2.06
19	12.801	[1aR (1aα, 4α, 4aβ, 7bα)]-1a,2,3,4,4a,5,6,7b-octahydro 1,1,4,7-tetramethyl-1H-Cycloprop[e]azulene	1410	0.19	± 0.005
20	12.957	Caryophyllene	1421	1.67	± 0.22
21	13.225	Aromandendrene	1440	0.65	± 0.032
22	13.437	Humulene	1456	0.24	± 0.002
23	13.538	Alloaromadendrene	1464	0.14	± 0.001
24	14.643	2-methoxy-4-(2-propenyl)- phenol acetate	1548	0.58	± 0.004

RT: Retention time, RI: Retention indices, SD: Standard deviation.

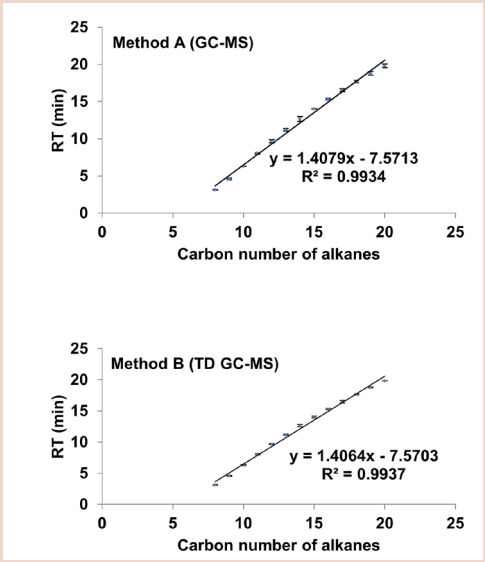

Figure 1: Carbon number of homologous alkane vs. retention time, the equations of the straight lines shows the linear relationships between both parameters for the method A and B.

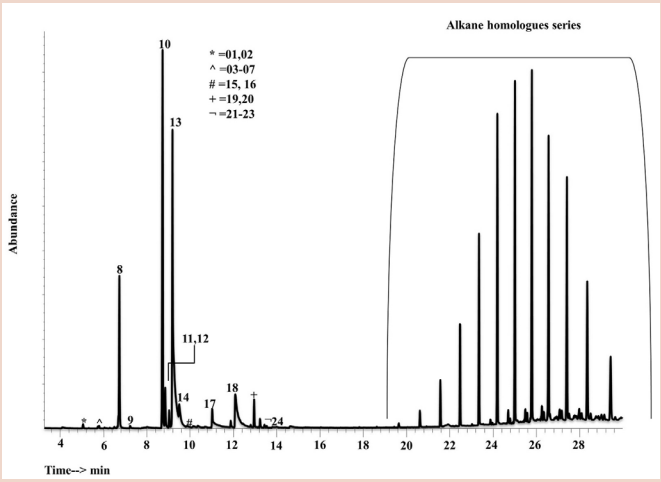

Figure 2: Total ion current gas chromatogram of volatile compounds of Tiger Balm® by GC-MS using a HP5-MS column (30 m×0.25 mm, i.d. 0.25 µm).

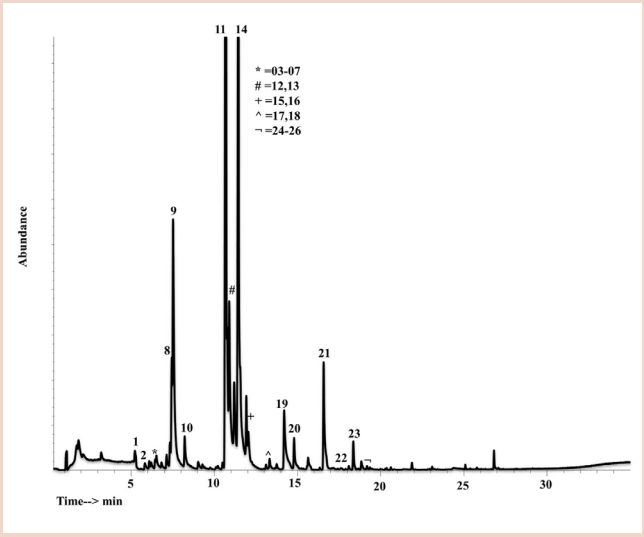

Figure 3: Total ion current gas chromatogram of volatile compounds of Tiger Balm® by TD GC-MS using a HP5-MS column (30 m×0.25 mm, i.d. 0.25 µm).

trap was then heated ballistically to 280°C and held for 3 min under a helium pressure of 10 psi and with an outlet split flow of 20 ml/min. The GC proceeded in an oven with temperature program: initial 60°C for 2 min, rose to 300°C at 10°C/min, and held at 300°C for 4 min under a constant pressure (10 psi). Following desorption, the volatiles were transferred through a deactivated glass capillary line at 200°C onto a HP5-MS column 5% phenyl-methylpolysiloxane, 30 m×0.25 mm, i.d.0.25 µm (Agilent Technologies, USA).

Alkanes standard

1 µl of n-alkane standard mixture was injected directly without dilution into the injection port and placed inside the desorption tube using method A and B, respectively.

RESULTS

Alkanes standard run by the two methods and the homologues alkane series show a linear relationship between the retention time (RT) and number of carbon atoms in the alkanes (Figure 1).

Direct analysis of TB hexane solution by GC-MS (method A) showed 24 compounds (Table 1) in addition to higher alkanes homologue series which are not counted as part of the total peak areas since they were resulted from the paraffin carrier (Figure 2).

Table 2: Volatile compounds identified and quantified in the headspaces of TIGER BALM® by TD-GC-MS

no	RT (min)	Compound name	RI	% ± SD (n=3)	
1	5.240	alpha-Pinene	933	0.82	± 0.07
2	5.586	Camphene	950	0.03	± 0.01
3	5.844	Benzaldehyde	963	0.32	± 0.01
4	6.217	beta-Pinene	981	0.28	± 0.02
5	6.835	alpha-Phellandrene	1009	0.30	± 0.09
6	7.146	(+)-4-Carene	1021	0.47	± 0.03
7	7.346	o-Cymene	1029	0.77	± 0.12
8	7.452	D-Limonene	1033	2.60	± 0.09
9	7.537	Eucalyptol	1036	11.30	± 0.09
10	8.229	gamma-Terpinene	1062	1.62	± 0.08
11	10.695	(+)-2-Bornanone (Camphor)	1153	26.89	± 1.91
12	10.899	Trans-5-methyl-2-(1-methylethyl)-Cyclohexanone (Trans-Menthone)	1160	7.45	± 0.69
13	11.201	Cis-5-methyl-2-(1-methylethyl)-Cyclohexanone (Cis-Menthone)	1171	3.72	± 0.25
14	11.463	menthol	1180	29.65	± 4.18
15	11.939	alpha-Terpineol	1197	2.21	± 0.11
16	12.063	Methyl salicylate	1202	2.26	± 0.07
17	13.333	Pulegone	1247	0.40	± 0.13
18	13.755	3-methyl-6-(1-methylethyl)-2-Cyclohexen-1-one	1262	0.17	± 0.01
19	14.217	(E)-Cinnamaldehyde	1278	3.29	± 0.06
20	14.808	[1S-(1α, 3β, 6α)]-3,7,7-trimethyl-Bicyclo[4.1.0]heptane	1299	1.16	± 0.06
21	16.585	Eugenol	1364	3.91	± 0.21
22	18.100	[1aR (1aα, 4α, 4aβ, 7bα)]-1a,2,3,4,4a,5,6,7b-octahydro 1,1,4,7-tetramethyl-1H-Cycloprop[e]azulene	1422	0.11	± 0.003
23	18.370	Caryophyllene	1434	0.71	± 0.07
24	18.566	Aromandendrene	1442	0.02	± 0.01
25	19.192	Humulene	1468	0.09	± 0.01
26	19.370	Alloaromadendrene	1476	0.09	± 0.007

Following the detection of the available standards by TD GC-MS, 26 volatile compounds were detected from TB by this method and the relative amount of individual compounds was expressed as percentage of each peak area relative to total peak areas (n=3) (Table 2). Camphor and menthol were detected with a large amount compared to other volatiles (Figure 3) and this is agreed with the constituents blend of TB stated by the manufacturer that it is composed of 11.0% natural camphor, 10.0% menthol, 5.0% clove oil, 7.0% cajuput oil, cinnamon oil, dementholised mint oil, yellow soft paraffin and hard paraffin.

DISCUSSION

Calculation of the RI under isothermal conditions is based on the retention times of homologues alkanes C_8-C_{20} and the sample components.[4] The RI is independent of column dimensions, and gas flow, but it is dependent of temperature programs, and stationary column polarity. This is why we need to run n-alkane standards each time when we change the method of sample analysis by GC-MS and TD GC-MS.

TB is a mixture of compounds which have different molecular masses and polarity and behave in a different manner toward the column material, hence we needed to design a temperature programs with a period of heating at a constant rate, which made the alkanes elution to be linear and not logarithmic.

TD GC-MS shows ability to resolves isomers from the head space without a further need to change column which indicates that it is a powerful technique for detection of volatile isomers. Volatile enantiomeric compounds in some plant-derived pharmaceuticals were successfully resolved by a capillary gas chromatography connected to dual flame-ionization detector using more than one type of column.[5] Most of volatiles detected by TD GC-MS were also detected by GC-MS, but TD GC-MS without using solvents, was more efficient in showing the 1:1 relative abundance of camphor and menthol which can be taken as a chemical marker of this herbal blend. In addition, dissolving 10 mg of TB in 1 ml hexane showed just nine compounds from GC-MS runs (results not shown), while dissolving 20 mg in 1 ml of hexane showed good signals. But we noticed the appearance of insoluble part of TB in hexane indicating the saturation of the solution, which is another reason encouraging us to use TD GC-MS as a quality control technique for TB.

CONCLUSION

TD GC-MS is a very useful technique for the analysis of volatile compounds from TB without the need of tedious pre-treatment with organic solvents; this technique could be applicable for quality assurance of any pharmaceutical preparation, as long as it releases aromatic essentials to the head space.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

Both authors have none to declare.

ABBREVIATION USED

TB: Tiger Balm®; **GC:** Gas Chromatography; **MS:** Mass Spectrometry; **GC-MS:** Gas Chromatography-Mass Spectrometry; **TD:** Thermal Desorption; **EI:** Electron Impact; **RI:** Retention Indices; **RT:** Retention Time.

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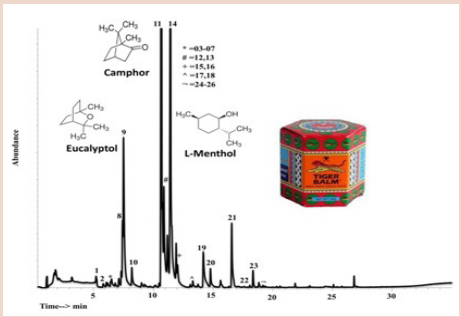
PICTORIAL ABSTRACT



SUMMARY

- In this work we applied efficiently thermal desorption gas chromatography mass spectrometry in quality control by analysis of volatile scents from a semisolid herbal medicine–Tiger Balm.
- This technique has advantage of avoiding tedious pre-treatment of samples with organic solvents, which analysts normally require when only GC-MS is used.

ABOUT AUTHORS

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