ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
17 (1998) 1427–1438

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Capillary gas chromatographic assay of camphor and *m*-cresol in dermatological creams[1]

Tahseen Mirza, Henry S.I. Tan *

Division of Pharmaceutical Sciences, College of Pharmacy, University of Cincinnati Medical Center, 3223 Eden Avenue, PO Box 67004, Cincinnati, OH 45267-0004, USA

Received 27 August 1997; accepted 5 February 1998

Abstract

Camphor and *m*-cresol mixtures are used in antiseptic and anti-itching creams. No compendial method exists for these preparations. This paper reports a capillary gas chromatographic method using FID detection with 2,6-di-*tert*-butyl-4-methylphenol as internal standard on a 30 m × 0.32 mm Supelcowax®-10 column (0.25 μm film) with helium as carrier gas. Ramped temperature programming was applied. The method allows simultaneous quantitation of camphor and *m*-cresol in the presence of *o*- and *p*-cresols, calamine and zinc oxide. Overall percent recoveries ($\pm$ SD, $n = 9$) of camphor, *o*-, *p*- and *m*-cresol from spiked placebo creams, at a labeled amount of 10 (w/w)% were 96.9 ± 0.6, 98.2 ± 0.6, 99.2 ± 0.5 and 101.0 ± 0.9%, respectively, and at a labeled amount of 1% were 96.7 ± 0.6, 97.8 ± 0.9, 97.8 ± 0.6, and 100.3 ± 1.0%, respectively. The recovery studies were carried out at $\pm$ 30% of the labeled amounts. Linear peak area or height ratios were obtained ($r > 0.999$) for camphor, *o*-, *p*- and *m*-cresol covering a concentration range of 10–200% of the labeled amount. Linearity ($r > 0.999$) was also obtained for *m*-cresol when the relative concentration of *o*- and *p*-cresol was varied from 5 to 100% of the *m*-cresol concentration. The resolution between the 'critical pair' of *p*- and *m*-cresol was ≥ 1.1. The limit of quantitation was 23 pg for *m*-cresol and 9.3 pg for camphor using an injection split of 1:50. The repeatability (%RSD) for all compounds were < 2% for peak area and < 1.4% for peak height ratios. System suitability and robustness of the method were established. The method was successively applied to the assay of available commercial products and allows assay of camphor and the three cresol isomers.

1. Introduction

Camphor and *m*-cresol mixtures are used in antiseptic and anti-itching creams. No compendial method exists for these preparations. However, methods have been cited in the literature for only the assay of cresols in pharmaceutical preparations. Sane et al. [1] have described a gas chromatographic method for the determination of *o*-, *p*- and *m*-cresol in pharmaceutical preparations using packed columns with a binary mixture of stationary phases. Chen et al. [2] assayed the cresols in Cresol USP and in Saponated Cresol

* Corresponding author. Tel.: +1 513 5583784; fax: +1 513 5584372.

[1] Presented to Analysis and Pharmaceutical Quality Section of the American Association of Pharmaceutical Scientists, 9th annual meeting, Miami Beach, FL, November, 1995.

0731-7085/98/$ - see front matter
PII S0731-7085(98)00032-6

Solution by reversed-phase high-performance liquid chromatography. *o*- and *p*-Cresol in the presence of benzylalcohol in industrial waste waters had been analyzed by gas chromatography [3]. Mixtures of camphor and one or more of the cresols and many terpenoids had been determined by gas chromatography in the essences of *L. graveolens*, *S. boliviana*, and *P. sagittalis* [4–6]. None of these published methods are applicable to the assay of camphor and *m*-cresol in pharmaceutical creams due to the complexity of the sample matrices and thus complicated sample work-up. In addition, these published methods did not assay a mixture of all four compounds (camphor, *o*-, *m*- and *p*-cresols) referred to in this work. Furthermore, the quantitative data were not complete.

This work describes the development and validation of a capillary gas chromatographic (CGLC) method for the simultaneous determination of camphor and *m*-cresol in the presence of zinc oxide, calamine, *o*- and *p*-cresols in dermatological creams. The method was successively applied to the assay of available commercial products.

2. Experimental

2.1. Apparatus

A Varian Model 3500 gas chromatograph (Varian Instruments, Walnut Creek, CA) equipped with a FID detector and a Varian model 8500 autoinjector was used in all the experiments. The injector was capable of making split-mode injections. Chromatograms were recorded and evaluated with Beckman PeakPro Rev. 2.2 Data acquisition system Las L.

2.2. Chemicals

Camphor, *o*-, *p*- and *m*-cresol, and 2,6-di-*tert*-butyl-4-methylphenol (DBMP) were obtained from Aldrich, Milwaukee, WI. Chloroform and methylene chloride (HPLC/GC grade) were obtained from EM Sciences, Gibstown, NJ. All other chemicals were of reagent grade.

2.3. GLC conditions

A 30 m × 0.32 mm (i.d.) Suplecowax-10 (Supelco, Bellefonte, PA) capillary gas chromatography column with 0.25 μm thick film was used. Helium was used as the carrier gas at 2.0 ml min^{-1} with nitrogen as the make-up gas at 30 ml min^{-1}. Ramped temperature programming was used for the successful elution of all the peaks of interest. The temperature programming comprised of holding the oven temperature at 140°C for 3 min, ramping to 170°C at 25°C min^{-1}, holding at 170°C for 10 min, ramping to 240°C at 30°C min^{-1} and finally holding at 240°C for 5 min. Injector and detector temperatures were 170 and 250°C, respectively. The injector volume was 1 μl and the injector split ratio was 1:50.

2.4. Preparation of placebo cream (greaseless cream base)

The oil phase of the cream was prepared by transferring 2.49 g of stearyl alcohol, 2.52 g of cetyl alcohol, 5.01 g of mineral oil, 2.39 g of Steareth®-20 and 2.41 g of Steareth®-2 into a stainless steel container. The contents were melted by heating at 65°C. The water phase was prepared by heating 100 g of water to 65°C in another stainless steel container. Then 0.52 g of Carbopol® was added to the warm water phase while stirring at 600 RPM with the aid of a mechanical stirrer. Finally, the oil phase was transferred into the water phase while stirring at 600 RPM for approximately 5 min. The contents were allowed to cool to room temperature and transferred into a plastic jar.

2.5. Preparation of placebo cream (greaseless cream base) containing zinc oxide and calamine

A greaseless cream base, containing zinc oxide and calamine, was prepared by heating 22 g of the above-prepared greaseless cream base to 65°C. Then 1.25 g of zinc oxide and 2.50 g of calamine was added to this warm base while stirring at 600 RPM. After cooling, this cream base was transferred into a plastic bottle.

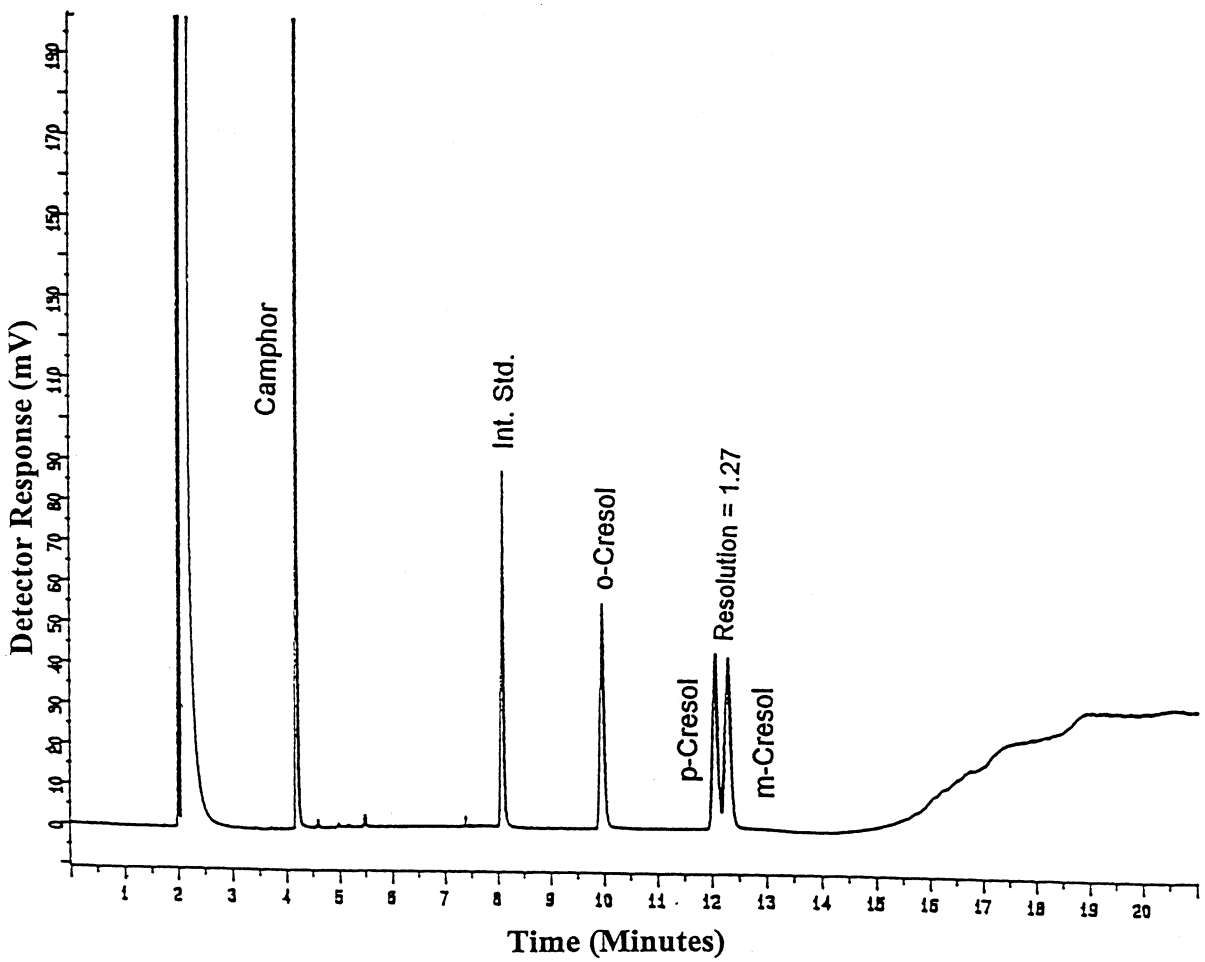

Fig. 1. Liquid chromatogram of a standard solution of camphor, *o*-, *m*-, *p*-cresol, and internal standard in methylene chloride.

2.6. Standard stock solutions

2.6.1. *o*-, *m*- and *p*-Cresol stock solutions

Aliquots of 500 μl of *m*- and *p*-cresol and 490 μl of *o*-cresol were each transferred with the aid of microsyringes into separate 100 ml volumetric flasks and diluted to volume with methylene chloride to obtain concentrations of 5.16 mg ml^{-1}. The concentrations were calculated using the density values of 1.0340, 1.0341, and 1.0470 of *m*-, *p*- and *o*-cresol, respectively.

2.6.2. Internal standard stock solution

A proportionate amount of DBMP was accurately weighed and dissolved in methylene chloride to obtain a concentration of 1 mg ml^{-1} of DBMP.

2.6.3. Composite stock solution

Approximately 300 mg of camphor was accurately weighed and transferred into a 100 ml volumetric flask. Into the same volumetric flask, 20.0 ml of *m*-cresol stock solution, and 4.0 ml of the *o*-, and *p*-cresol stock solutions were pipetted and diluted to volume with methylene chloride. The composite stock solution thus contained 3.0000 mg ml^{-1} of camphor, 1.0320 mg ml^{-1} of *m*-cresol and 0.2060 mg ml^{-1} of *o*- and *p*-cresols.

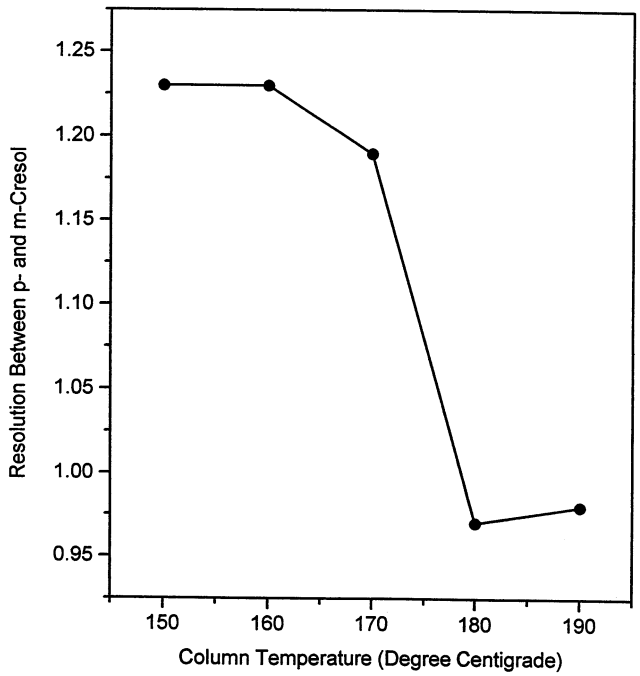

Fig. 2. Graphical presentation of column temperature (second step in temperature program) versus resolution between the critical pair of *p*- and *m*-cresol.

2.7. Preparation of calibration standards

2.7.1. Calibration standards containing varying amounts of camphor, o-, p- and m-cresol

A set of standard solutions containing camphor, *o*-, *p*- and *m*-cresol covering the entire concentration range of the actives in the commercial products were prepared. Using appropriate-sized pipettes, aliquots of the composite stock solution were transferred into separate 100 ml volumetric flasks. Into each flask, 5.0 ml of the internal standard stock solution was added and diluted to volume with methylene chloride. Each flask thus contained the varying concentrations of camphor, *o*-, *p*- and *m*-cresol and a constant concentration of the internal standard (ca 50 μg ml^{-1}).

2.7.2. Calibration standards containing varying amounts p-cresol in the presence of a constant amount of m-cresol

A set of six standard solutions were prepared by pipetting 1.0 ml of *m*-cresol stock solution which was further diluted 10-fold and 5 ml of the internal standard stock solution into six separate 100 ml volumetric flasks. Then using appropriate sized pipettes, aliquots of a *p*-cresol stock solution were pipetted into the six flasks. The flasks were diluted to volume with methylene chloride. Each solution thus contained ca 52 μg ml^{-1} of *m*-cresol and 50 μg ml^{-1} of the internal standard. The concentration of *p*-cresol in these solutions varied from 5 to 100% with respect to the concentration of *m*-cresol.

2.7.3. Calibration standards containing varying amounts m-cresol in the presence of a constant amount of p-cresol

A set of six standard solutions were prepared by following the procedure described in the previous section except that the concentration of *p*-cresol was kept constant while the concentration of *m*-cresol was varied. The concentration of *m*-cresol in these solutions varied from 5 to 100% with respect to the concentration of *p*-cresol.

Table 1
Linearity of camphor, *o*-, *p*- and *m*-cresol

Std.	Concentration (μg ml^{-1})				Peak area ratios			
	Camphor	*o*-Cresol	*p*-Cresol	*m*-Cresol	Camphor	*o*-Cresol	*p*-Cresol	*m*-Cresol
(a) Concentrations corresponding to the label claim of 1% (w/w)								
1	1.2	2.1	2.1	—	0.0210	0.0365	0.0354	—
2	2.4	4.1	4.1	—	0.0414	0.0753	0.0742	—
3	3.5	6.2	6.2	—	0.0597	0.1089	0.1111	—
4	5.9	10.3	10.3	—	0.1011	0.1820	0.1763	—
5	9.4	16.5	16.5	—	0.1628	0.2905	0.2796	—
6	11.7	20.6	20.6	—	0.2021	0.3727	0.3531	—
Correlation coefficient (*r*)					0.9999	0.9997	0.9998	
Slope (*m*)					0.0173	0.0179	0.0169	
y-Intercept (*b*)					0.0004	−0.0010	0.0032	
(b) Concentrations corresponding to the label claim of 10% (w/w)								
1	32.3	10.3	10.3	10.3	0.5868	0.1866	0.1786	0.1782
2	64.5	20.6	20.6	20.6	1.1909	0.3802	0.3626	0.3520
3	96.8	31.0	31.0	31.0	1.8192	0.5744	0.5439	0.5235
4	161.3	51.6	51.6	51.6	3.0752	0.9286	0.9014	0.8782
5	258.1	82.6	82.6	82.6	5.0203	1.5398	1.4711	1.4121
6	322.6	103.2	103.2	103.2	6.4111	1.9227	1.8569	1.7915
Correlation coefficient (*r*)					0.9998	0.9998	0.9999	0.9999
Slope (*m*)					0.0200	0.0187	0.0180	0.0173
y-Intercept (*b*)					−0.1042	−0.0099	−0.0135	−0.0079

—, *m*-Cresol not added to the standards.

2.8. Sample preparations

2.8.1. For recovery studies

The spiked samples were prepared by weighing ca 250 mg of the greaseless base into separate 100 ml volumetric flasks. After the addition of 5.0 ml of the internal standard solution, the appropriate volumes of a stock solution containing camphor, *o*-, *p*- and *m*-cresol were transferred into each flask. The flasks were diluted to volume with methylene chloride and sonicated for approximately 5 min. The sample solutions were filtered through Acrodisc® PTFE filter cartridges, discarding the first 3 ml.

2.8.2. For commercial products

About 300 mg of the cream was accurately weighed into a glass weighing boat. The cream was transferred into a 100 ml volumetric flask by flushing the glass weighing boat with methylene chloride. After 5.0 ml of the internal standard stock solution was pipetted into the volumetric flask, the mixture was diluted to volume with methylene chloride. After sonication for 5 min, the sample solution was filtered through a PTFE filter cartridge. The first 3 ml of the filtrate were discarded and an aliquot of the filtrate was collected into a GC vial.

2.9. Chromatographic procedure

Exactly 1.0 ml aliquots of the calibration standard solutions and the sample solutions were injected separately into the GC using the optimized chromatographic conditions, and the chromatograms were recorded. The amount of camphor and *m*-cresol in the samples were determined by comparing the drug/internal standard peak area ratios to those of the respective standard solutions.

Table 2
Injection repeatability on two different days using peak area and peak height ratios

Day	Mean peak area ratios[a]				%RSD of peak area ratios			
	Camphor	*o*-Cresol	*p*-Cresol	*m*-Cresol	Camphor	*o*-Cresol	*p*-Cresol	*m*-Cresol
1	4.7733	0.2750	0.2565	0.8733	0.08	1.21	2.39	1.70
2	4.8190	0.2746	0.2575	0.8770	0.63	1.06	1.61	1.00
Overall[b]	4.7962	0.2748	0.2570	0.8752	0.36	1.14	2.00	1.35
	Mean peak height ratios[a]				% RSD of peak height ratios			
	Camphor	*o*-Cresol	*p*-Cresol	*m*-Cresol	Camphor	*o*-Cresol	*p*-Cresol	*m*-Cresol
1	8.5881	0.1761	0.1360	0.4355	1.00	0.78	1.64	2.04
2	8.4022	0.1761	0.1396	0.4479	0.50	0.95	1.01	0.54
Overall[b]	8.4952	0.1761	0.1378	0.4417	0.75	0.87	1.33	1.29

[a] The concentrations of camphor, *o*-, *p*- and *m*-cresol corresponded to the product containing 10% (w/w) of *m*-cresol and camphor.
[b] $n = 10$.

3. Results and discussion

3.1. Selection of internal standard

A number of compounds, including *p*-xylene, phenol, tetradecanol, benzilic acid, and DBMP were injected under the CGLC conditions to determine if they could serve as an internal standard. Results indicated that DBMP was the best choice to serve as an internal standard because its retention time was longer than that of camphor but shorter than that of *o*-, *m*- and *p*-cresol. When injected along with camphor, *o*-, *m*- and *p*-cresol, DBMP exhibited a well resolved peak that elutes right in the middle of the chromatogram. The concentration of the internal standard was chosen to be exactly as that of *m*-cresol at the 100% label amount of the 10(w/w)% formulation. A typical chromatogram of a standard solution containing the internal standard is shown in Fig. 1.

3.2. Development of the analytical method

3.2.1. Optimization of column temperature

Results from initial studies demonstrated that a three-step column temperature program was necessary in order to get the desired separation between the peaks of interest, methylene chloride and the excipients. Initially, in the first step, the column temperature was held at 140°C for 3 min to elute the methylene chloride solvent. The column temperature was then raised to 195°C and held at that temperature for 10 min. During this

Table 3
Recoveries of camphor, *o*-, *p*-, and *m*-cresol from spiked placebo without zinc oxide and calamine (label claim 10% w/w camphor/*m*-cresol)

% Label claim	Mean % recoveries ($n = 3$)				S.D. Of % recoveries ($n = 3$)			
	Camphor	*o*-Cresol	*p*-Cresol	*m*-Cresol	Camphor	*o*-Cresol	*p*-Cresol	*m*-Cresol
70	96.4	98.0	99.1	101.5	0.5	0.2	0.2	0.8
100	96.8	98.0	99.1	100.8	0.1	0.2	0.4	0.6
130	97.4	98.9	99.3	101.2	0.8	1.0	1.0	1.6
Overall mean[a]	96.9%	98.2%	99.2%	101.0%	0.6%	0.6%	0.5%	0.9%

[a] $n = 9$.

Table 4
Recoveries of camphor, *o*-, *p*-, and *m*-cresol from spiked placebo containing zinc oxide and calamine (label claim 1% w/w camphor/*m*-cresol)

% Label claim	Mean % recoveries ($n = 3$)				S.D. of % recoveries ($n = 3$)			
	Camphor	*o*-Cresol	*p*-Cresol	*m*-Cresol	Camphor	*o*-Cresol	*p*-Cresol	*m*-Cresol
70	96.7	98.4	97.6	100.9	0.9	0.6	1.1	0.9
100	96.6	97.4	98.0	99.6	0.6	1.2	0.1	1.3
130	96.7	97.6	97.9	100.4	0.6	0.8	0.6	1.0
Overall mean[a]	96.7	97.8	97.8	100.3	0.6	0.9	0.6	1.0

[a] $n = 9$.

step, the internal standard eluted first, followed by *o*-, *p*- and *m*-cresol, respectively. However, the resolution between *p*- and *m*-cresol was unsatisfactory. In the final step, the column temperature was raised to 240°C. At this temperature all the excipients eluted within 5 min. In order to optimize the 'critical resolution' between *p*- and *m*-cresol, a systematic study was conducted by varying the temperature in the middle step of the temperature program. A graphical presentation of column temperature versus resolution between *p*- and *m*-cresol is presented in Fig. 2. The results show that the 'critical resolution' was ca 1.2 or better when the column temperature in the middle step was 150, 160 and 170°C but decreased sharply to 0.97 at 180°C. The retention factor, k', of *m*-cresol was 11.5 at 150°C and decreased gradually with increasing column temperatures. The graph showed that 160 or 170°C could be used as the middle step temperature in the temperature program. At these temperatures, the k' value of *m*-cresol was < 10 and the 'critical resolution' between *p*- and *m*-cresol was ca 1.2.

3.2.2. Optimization of carrier gas flow rate

From the previous study, 160 or 170°C was

Table 5
Youden's experimental design for robustness studies

Variables	Experiment #							
	1	2	3	4	5	6	7	8
Col. temp. (°C)	A	A	A	A	a	a	a	a
Flow (ml min^{-1})	B	B	b	b	B	B	b	b
Inj. temp. (°C)	C	c	C	c	C	c	C	c
Column	D	D	d	d	d	d	D	D
Split	E	e	E	e	e	E	e	E
Det. temp. (°C)	F	f	f	F	F	f	f	F
Solvent	G	g	g	G	g	G	G	g
Col. temp. (° C)	173	173	173	173	167	167	167	167
Flow (ml min^{-1})	2.2	2.2	1.8	1.8	2.2	2.2	1.8	1.8
Inj. temp. (°C)	173	167	173	167	173	167	173	167
Column	Old	Old	New	New	New	New	Old	Old
Split	1:55	1:45	1:55	1:45	1:45	1:55	1:45	1:55
Det. temp. (°C)	255	245	245	255	255	245	245	255
Solvent[a]	MEC	CHL	CHL	MEC	CHL	MEC	MEC	CHL
Observed result	s	t	u	v	w	x	y	z

[a] MEC, methylene chloride; CHL, chloroform.

Table 6
Differences obtained between the two levels of each variable described in the Youden's robustness test

Variable	Difference in % recovery of *m*-cresol		
	Low level 31 (μg ml^{-1})	High level (72 μg ml^{-1})	Std. curve correl. coeff. (*r*)
Column age	0.3	1.6	0.0000
Detector temperature	0.2	1.2	0.0000
Injector split Ratio	0.6	1.3	0.0000
Solvent type	0.4	1.0	0.0000
Column temperature	0.2	0.7	0.0001
Injector temperature	0.7	0.4	0.0000
Carrier gas flow rate	0.2	0.3	0.0001
S.D.	0.6	1.5	—

identified as the column temperature for the middle step of the column temperature programming. The purpose of this study was to optimize the carrier gas flow rate at each of the aforementioned column temperatures and then select the optimum conditions from the combined results of the optimization studies. In this study, a standard solution was injected onto the column at carrier gas flow rates of 1.0, 1.5 and 2.0 ml min^{-1}, respectively, at each of the two column temperatures. As expected, the resolution between *p*- and *m*-cresol for the three flow rates was slightly better at a column temperature of 160°C than at 170°C. However, the k' values were considerably lower at 170°C than at 160°C. Since the resolutions were not significantly different at the two column temperatures but the capacity factors were significantly lower at 170°C, it was chosen as the column temperature for the second step of the temperature program. The carrier gas flow rate of 2 ml min^{-1} was chosen as the flow rate for the same reasons.

Table 7
Assay results of the commercially available dermatological creams

Pouch no	Replicate	Product A[a]			Product B[b]		
		% (w/w)		Total %	% (w/w)		Total %
		Camphor	*m*-Cresol		Camphor	*m*-Cresol	
1	1	0.24	0.81	1.05	6.05	2.37	8.42
	2	0.26	0.86	1.13	6.05	2.34	8.39
	3	0.23	0.86	1.08	6.07	2.32	8.39
2	1	0.27	0.86	1.13	6.03	2.32	8.34
	2	0.22	0.86	1.07	6.07	2.34	8.40
	3	0.27	0.88	1.15	6.07	2.33	8.40
3	1	0.24	0.84	1.08	6.11	2.35	8.46
	2	0.23	0.79	1.02	6.05	2.34	8.39
	3	0.21	0.85	1.06	6.08	2.35	8.44
Overall mean[b]		0.24%	0.85%	1.09%	6.06%	2.34%	8.40%
Overall S.D.[b]		0.02%	0.03%	0.04%	0.03%	0.01%	0.03%

[a] Product A also contains zinc oxide and calamine.
[b] $n = 9$.

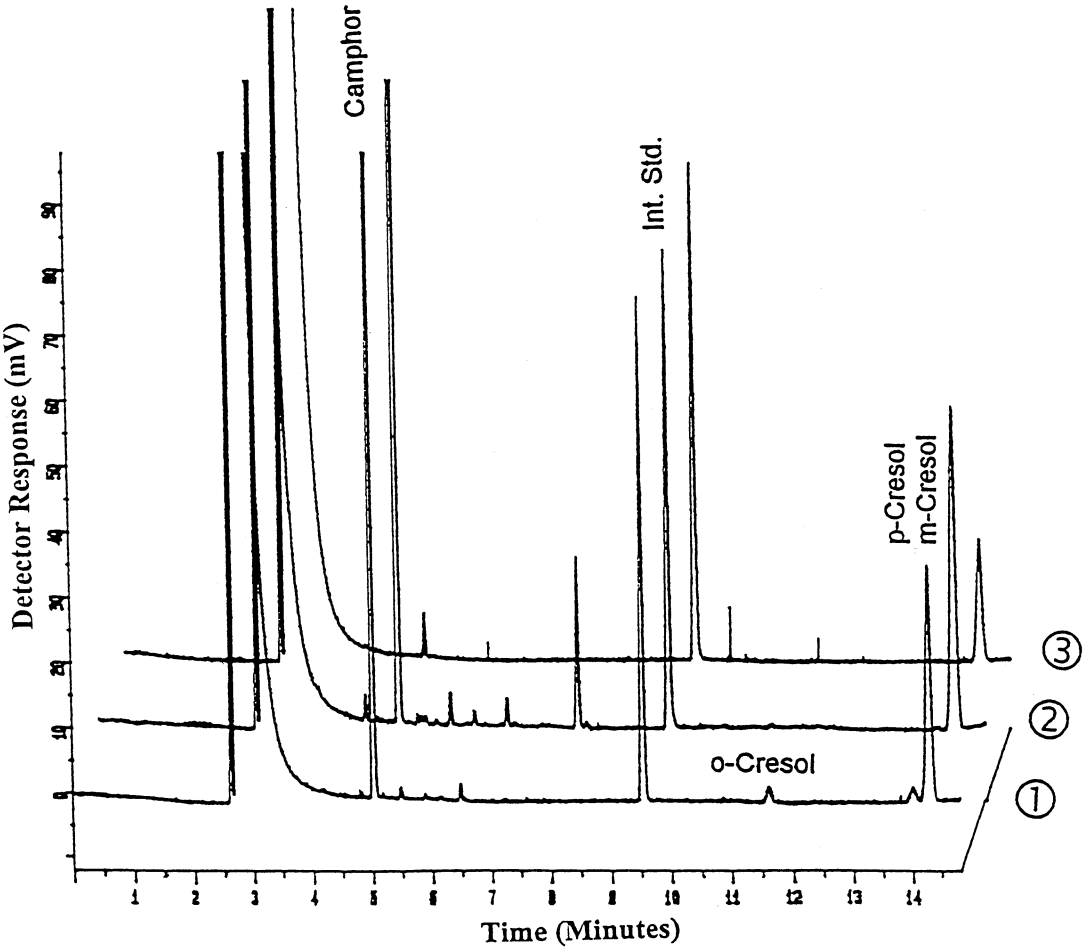

Fig. 3. Liquid chromatograms of methylene chloride solutions of mixture of standards (1), ®commercial product A (does not contain zinc oxide and calamine) (2) and commercial product B (contains zinc oxide and calamine) (3).

3.3. Assay validation

3.3.1. Linearity

Linearity of camphor, o-, p- and m-cresol covering the concentration range in the commercial products.

The linear regression data for peak area ratios versus concentration of camphor, *o*-, *p*-and *m*-cresol demonstrated that the detector response for the four compounds was linear over the concentration range required for the analysis of the commercial products (Table 1). The correlation coefficient, *r*, for all four compounds was better than 0.999. Because of the expected wide ranges in concentration of camphor, and perhaps of *o*- and *p*-cresol in the formulations, it was necessary to prepare two sets of standard solutions for these compounds. On the basis of the precision results described in Section 3.3.3, subsequent studies utilized peak area data. Results obtained from the analysis of the two sets of calibration standards corresponding to two different concentration levels in the commercial products are presented in Table 1.

3.3.1.1. Linearity of p-cresol in the presence of constant amount of m-cresol. Because *p*- and *m*-cresol do not show baseline resolution, a study was undertaken to determine if the drug/internal detector response ratio of each isomer is linear with concentration in the presence of varying

amounts of the other isomer. The linearity for *p*-cresol was determined by varying its concentration in the presence of a constant amount of *m*-cresol. The correlation coefficient obtained from the linear regression of peak area ratio versus concentration of *p*-cresol was 0.9997. In this study, the concentration of *p*-cresol was varied from 5 to 100% in relation to the concentration of *m*-cresol. It is thus concluded that the detector response for *p*-cresol was linear over the relative concentration range of 5–100% of that of *m*-cresol when its concentration was kept constant at ca 52 μg ml^{-1}.

3.3.1.2. Linearity of m-cresol in the presence of a constant amount of p-cresol. The linearity of *m*-cresol was determined by varying its concentration from 5 to 100% of *p*-cresol concentration that was kept constant at 52 μg ml^{-1}. The correlation coefficient obtained from the linear regression analysis of peak height ratio versus concentration of *m*-cresol was 0.9998. This indicates that the detector response for *m*-cresol was linear over the relative concentration range of 5–100% of that of *p*-cresol.

3.3.2. Limit of quantitation (LOQ)

The extrapolation method of Glaser and Forest [7] was used in this study for the determination of LOQ with three concentrations of camphor and *m*-cresol near LOQ, whereby each concentration was injected seven times. The S.D. of the peak areas ratios at each concentration were plotted against the corresponding concentration. Linear regression of the S.D. versus concentration plot gave a *Y*-intercept, So, that upon multiplying by 10 gave the minimum response limit [8]. A standard solution containing *m*-cresol at higher concentration was prepared and injected into the GC five times under the optimized conditions. From the average peak area ratio of this standard, the concentration of *m*-cresol that would give a response of 10∗So was determined to give the LOQ. In this manner, the LOQ for *m*-cresol was calculated to be 23 pg of *m*-cresol injected and 9.3 pg for camphor injected. The % RSD of the peak area ratios at the LOQ level were better than 3.5%.

3.3.3. Injection repeatability

The results obtained from the replicate injections on two different days of a standard solution containing *m*-cresol and camphor at the labeled amount of a commercial product and also containing *o*- and *p*-cresol at very low concentration are presented in Table 2. The results indicate that the peak area ratio method is more sensitive for *o*-, *p*- and *m*-cresol while the peak height ratio method is more sensitive for camphor. The results also demonstrate that at an average, the reproducibility was similar for *m*-cresol for both peak area and peak height ratios. For *o*- and *p*-cresol the reproducibility by peak area ratio was slightly better. The overall % RSD for all the components for both peak area and peak height was $\leq 2\%$. Based on these findings, the peak area ratio method was used in subsequent studies. A one-way ANOVA test revealed that there was no significant difference at the 95% confidence level for between-days precision of all the four components.

3.3.4. Recovery studies

3.3.4.1. Recovery studies using spiked placebo without zinc oxide and calamine (label claim 10 (w/w)% camphor/m-cresol). (a) *o*- and *p*-Cresol spiked at very low concentrations: The results obtained from the assays of the placebo spiked with camphor and *m*-cresol (label claim 10 (w/w)% camphor/*m*-cresol) that also contained *o*- and *p*-cresol at low levels are tabulated in Table 3. The results demonstrate that the percent recovery of all the four components was $>96\%$ with S.D. of $<1\%$. The experiments were repeated on the next day. The data indicated that the recoveries for *o*- and *p*-cresol were almost identical on both days while the recoveries of camphor and *m*-cresol was slightly lower on the second day.

(b) *o*- and *p*-Cresol spiked at high concentrations: These recovery studies were repeated in order to determine if *o*- and *p*-cresol present at the same level as *m*-cresol had any effect on the recoveries. The results indicated that *o*- and *p*-cresol, if present at the same level as *m*-cresol, do not affect the recoveries of any of the components. The recoveries of all the four components

were $>95\%$. The overall % RSD ($n=9$) of the recoveries of each of the four component were $<1\%$.

Recovery studies using spiked placebo containing zinc oxide and calamine (label claim 1 (w/w)% camphor/*m*-cresol): The results obtained from the analysis of the placebo containing zinc oxide and calamine spiked with camphor and *m*-cresol (label claim 1 (w/w)% camphor/*m*-cresol) and also containing *o*- and *p*-cresol at the same concentration as *m*-cresol, are tabulated in Table 4. The results show that the mean % recoveries ($n=9$) of all the four components was $>96\%$ and the S.D. ($n=9$) was $\leq 1\%$.

3.3.5. Robustness studies

The ruggedness test was conducted with *m*-cresol because, compared to camphor, its peak is much smaller than that of camphor. Also, *m*-cresol is not quite baseline-resolved from *p*-cresol. Youden and Steiner's experimental design [9] is presented in Table 5. The design is based on $(n+1)$ measurements to test the effect of n variables in order to determine if slight variations in daily use from the proposed optimized conditions will have any effect on the assay results. The seven variables and the two levels of each variable chosen are shown in Table 5. The observed results to be evaluated were percent recoveries from the spiked placebo solutions prepared at two levels of *m*-cresol at 31 μg ml^{-1} (low level) and 72 μg ml^{-1} (high level) and the correlation coefficient (r) of the standard curve. The results were designated by letters s through z. In order to find out for instance, if the column temperature had any effect on the results, the average of s, t, u and v is compared with the average of w, x, y and z. This distribution gives two groups of four determinations and each group contains the other six factors twice at the high level and twice at the lower level. The effects of all the other six factors cancel out except of that for changing the column temperature from high (A) to low (a).

The differences obtained between the two levels of each variable are ranked in decreasing order in Table 6. The results indicate that perhaps column age is the most important variable and has the most effect on the results especially at a high concentration level of *m*-cresol. For the newer column with high efficiency, the SD at the high level (72 μg ml^{-1}) dropped from 1.5 to 1.2, a 20% decrease. However, the column age did not have any significant effect at the lower level (31 μg ml^{-1}) of *m*-cresol. The other variables did not have much effect on the results as evidenced from the relatively low S.D.s observed among the differences in the two levels of the variables. Hence, it is concluded that the method was very robust when subjected to slight variations in the variables as would be expected if the method were to be used routinely.

The percent recovery values obtained at each concentration were compared. Analysis of the data by the *F*-test revealed that the differences in the recoveries at the two levels were not significant.

3.3.6. Selectivity

The selectivity of this method was determined by injecting blank placebo solutions into the GC under the proposed conditions. Another chromatogram was obtained for the placebo matrix solution containing zinc oxide and calamine. There were no interfering peaks in either chromatogram to suggest co-elution with the peaks of interest.

3.3.7. System suitability

In order to ensure successful resolution of the peaks of interest and to ensure that the system has acceptable precision, the method is required to meet certain system suitability criteria. It is recommended that a standard solution containing *m*-cresol and camphor corresponding to the label amount be injected into the GC. The standard must also contain *o*- and *p*-cresol at the same concentration as that of *m*-cresol. The system suitability criteria for this method were chosen by thoroughly analyzing the entire data collected during the method development and validation studies. Based on the results, the tailing factor for all the peaks of interest should be <1.5. The resolution between *p*- and *m*-cresol 'critical resolution' should be at least 1.2. If these criteria are met, it is recommended that the standard solution is injected five times into the GC and the %RSD

of the peak area ratio of each of the peaks should be $< 1.5\%$.

Finally, a set of calibration standard solutions whose preparation is described in the experimental section should be injected into the GC. The correlation coefficient (r) of the peak area ratios versus concentration for each component should be at least 0.999.

3.4. Assays of commercial products

The available commercial products labeled as product A and product B were assayed by the proposed method. The assay results are tabulated in Table 7. Typical chromatograms of the commercial products are presented in Fig. 3. The label claims of these commercial products are not very clear and attempts to obtain more information from the manufacturers were not successful.

Acknowledgements

Dr Randall Wickett (Cosmetics Science program, College of Pharmacy, University of Cincinnati Medical Center) is acknowledged for providing the raw materials and for consulting in the preparation of the placebo cream bases.

References

[1] R.T. Sane, K.K. Sonawane, Indian Drugs 25 (10) (1988) 420–424.
[2] C.H. Chen, C.H. Chiang, Chung-hu Yao Hseuh Tsa Chih 40 (2) (1988) 85–91.
[3] Y.I. Korenman, V.A. Minasyants, V.N. Fokin, Zh. Anal. Khim. 43 (1988) 1303–1306, C.A. 109, 115755.
[4] E.C. DeRiscala, J.A. Retamar, Arch. Bioquim, Quim. Farm. 18 (1973) 25–28.
[5] S.P. Tomasini, J.A. Retamar, Arch. Bioquim, Quim. Farm. 18 (1973) 7–14.
[6] E.C.J. Talenti, Essenze Deriv. Agrum., 52 (1982) 21–51; C.A. 99: 145934.
[7] J.A. Glaser, D.L. Forest, Environ. Sci. Technol. 15 (12) (1981) 1426.
[8] US-FDA, Center Drug Eval. Res., Fed. Reg., 61 (1996) 9318, US Gov. Printing Off. Washington, DC.
[9] W.J. Youden, E.H. Steiner, Statistical Manual of the AOAC, Association of Official Analytical Chemists, Arlington, VA, 1975, p. 33.