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ORIGINAL RESEARCH ARTICLE

Determination of Benzocaine, Chlorbutol, P-Dichlorobenzene and A-Pinene in Pharmaceutical Preparation by Gas Chromatography with Flame Ionization Detector

Navdeep Saini[*1], Koyal Saini[1] and Dr.B. P.Nagori[2], Dr. G.K. Singh[2], Sudhir Pandya[3]

[1]*Department of Quality Assurance, Mandsaur Institute of Pharmacy, Rewas Dewada Road, Mandsaur, Madhya Pradesh, India.*
[2]*Department of Quality assurance, Lachoo Memorial College of Pharmacy, Jodhpur, Rajasthan, India.*
[3]*Head, Dept of Quality Assurance, Nulife pharmaceutical Ltd., Pune (M.H.), India*

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ABSTRACT

A simple and rapid method for determination of Benzocaine, chlorbutol, paradichlorobenzene and α-pinene in pharmaceutical preparations was developed and validated using gas chromatography with flame ionization detection (GC-FID). GC separation was performed in about 12 min using a 5% SE-30 column (6ft ×1/8 inch). Nitrogen was used as carrier gas at a flow-rate of 2 ml min^{-1}. After injection of the sample at inlet temperature 225 ^{0}C, the temperature of GC oven was as follows: initial temperature was 150 ^{0}C, held for 1 min, increased to 180 ^{0}C at 50 ^{0}C min^{-1} held for 1 min, and finally to 250^{0}C at 50^{0}C min^{-1} with a final hold of 1.5 min. Calibration curves of benzocaine, chlorbutol, p-dichlorobenzene and α-pinene were linear between the concentration range of 100 to 1000µgml^{-1}, 200 to 2000µgml^{-1}, 32 to 320µgml^{-1} and 1.2 to 12µgml^{-1} respectively. The method was found to be specific, precise and accurate. The method was applied for the quality control of commercial ear drop containing Benzocaine, chlorbutol, paradichlorobenzene and α-pinene to quantify the drug and to check the formulation content uniformity.

Key words: GC, FID, α-pinene, Benzocaine, Chlorbutol, P-dichlorobenzene.

INTRODUCTION

Benzocaine is a widely used local and topical anesthetic. Benzocaine ear drops are used for temporary relief of ear pain. It do not treat an ear infection and therefore antibiotics are needed if ear pain is due to infection [1,2]. Chlorbutol[3] and paradichlorobenzene[4] have antibacterial and antifungal action. α-pinene[5] is a rubifacient. Formulation containing benzocaine, chlorbutol, paradichlorobenzene and α-pinene is used in ear blockage due to wax production in ears. It helps to dissolve the ear wax in the ear. Extensive literature survey reveals that no method reported for the simultaneous determination of benzocaine, chlorbutol, p-dichlorobenzene and α-pinene from its pharmaceutical formulation. The present research work describes a GLC [6] method for estimation of benzocaine, chlorbutol, p-dichlorobenzene and α-pinene from its pharmaceutical formulation using nutmeg oil as an internal standard. Gas chromatography was equipped with standard oven option for temperature ramping, split/splitless injection ports and flame ionization detector, 5% SE-30 column (6ft * 1/8 inch id max. temp. 300°C). The detector used was flame ionization detector with nitrogen as carrier gas in the split mode by direct injection method was used. The observations of method were subjected to statistical validation to determination for its accuracy and precision [7].

MATERIALS AND METHODS

Chemicals and Reagents

Benzocaine, chlorbutol, p-dichlorobenzene and α-pinene were obtained from Ramdev Chemicals, Polydrug Laboratories, Aarti Industries Limited and Panachem Organics, India respectively. Methanol (HPLC Grade) was purchased from Loba Chemie (New Delhi), and other chemicals and solvents used were of analytical grade. Ear drops preparation containing was obtained from local pharmacy.

Instrumentation

The GC-FID method was performed an CHEMICO CERES-800 PLUS GC operated with a split injector and equipped with a flame ionization detector, Agilent chemstation and 5%

***Corresponding Author:** Saini Navdeep, **Email:** saininavdeep079@gmail.com, **Phone No:** +91-9589796160

SE-30 (6ft * 1/8 inch id max. temp. 300^0C) stainless steel packed column, coated with 5% SE on 80/100 mesh Cromosorb WHP solid packed.. Injection and detector temperature are 225 ^{0}C and 250 ^{0}C, respectively. The carries gas (N_2) flow-rate was kept constant during the run at 2 ml min^{-1}. Nitrogen (30 ml min^{-1}), Hydrogen (35 ml min^{-1}) and synthetic air (350 ml min^{-1}) were used as auxiliary gases for the flame ionization detector.

Preparation of Standard

The stock standard solution of benzocaine, chlorbutol, p-dichlorobenzene and α-pinene were prepared in methanol to a concentration of 1000µgml^{-1}, 2000µgml^{-1}, 320µgml^{-1} and 12µgml^{-1}. Working standard solutions were prepared from the stock standard solutions. The calibration graphs were constructed in the range of 100 to 1000µgml^{-1}, 200 to 2000µgml^{-1}, 32 to 320µgml^{-1} and 1.2 to 12µgml^{-1} for benzocaine, chlorbutol, p-dichlorobenzene and α-pinene. For quality control samples containing concentration 3, 5, 7 mg ml^{-1} of mexiletine, the stock solution was diluted with methanol.

Benzocaine, chlorbutol, p-dichlorobenzene and α-pinene were prepared by diluting with methanol. The standard stock solution containing 1000µgml^{-1}, 2000µgml^{-1}, 320µgml^{-1} and 12µgml^{-1} of benzocaine, chlorbutol, p-dichlorobenzene and α-pinene respectively. From these stocks 10 serial working standard solutions were prepared to obtained concentration ranging from 100 to 1000µgml^{-1} ,200 to 2000µgml^{-1} ,32 to 320µgml^{-1} and 1.2 to 12µgml^{-1} for benzocaine, chlorbutol, p-dichlorobenzene and α-pinene respectively, volume was made with methanol . 1mcl of working standards were injected in to gas chromatograph and standard calibration curves were obtained for benzocaine, chlorbutol, p-dichlorobenzene and α-pinene.

Procedure for pharmaceutical preparation

Accurately weighed 3gm sample(Ear Drop) in a 50ml volumetric flask added to it 50ml 2% Nutmeg oil solution in methanol (Nutmeg oil is used as an internal standard) close and clamp the volumetric flask with the help of stopper and shake the flask with the help of wrist shaker for 3hr filter the contents through anhydrous sodium sulphate using whatmann filter paper No. 1, From these samples 1mcl samples were injected and analyzed by GC-FID for the concentrations of benzocaine, chlorbutol, p-dichlorobenzene and α-pinene.

RESULTS AND DISCUSSION

Method development and optimization

During method development, the injection port and detector temperatures were set to 225^0C and 250^0C, respectively. Different temperature programs were investigated to give an optimum temperature program as follows; initial temperature was 150^0C, held for 1 min, increased to 180^0C at 50^0C min^{-1} held for 1 min and finally to 250^0C at 30^0C min^{-1} with a final hold of 1.5 min. The injector volume was 1µl in splitless mode. The retention time for benzocaine, chlorbutol, paradichlorobenzene and α-pinene was found to be 3.827, 11.603, 4.780, 6.66 min respectively with good peak shape. No further optimization of the method was required. Additionally, preliminary precision and linearity studies performed during the development of the method showed that the 1µl injection volume was reproducible and the peak response was significant at the analytical concentration chosen. Typical chromatograms obtained with standard benzocaine, chlorbutol, paradichlorobenzene and α-pinene and ear drop are presented in (Fig 1,2,3,4, 5 & 6).

METHOD VALIDATION

Linearity

The linearity of peak area response versus concentration for benzocaine, chlorbutol, p-dichlorobenzene and α-pinene was studied over concentration range of 100 to 1000µgml^{-1} ,200 to 2000µgml^{-1} ,32 to 320µgml^{-1} and 1.2 to 12µgml^{-1} respectively. The calibration curve constructed was evaluated by its correlation coefficient. The correlation coefficients (*r*) of all the calibration curves were equal to 0.999. Standard deviations of the slope and intercept for the calibration curves were in (**Table 1**)

Precision and accuracy

The precision of GC-FID method was determined by repeatability (within-day) and intermediate precision (between-day). Three different concentrations which were quality control samples (3, 7, 11 mgml^{-1}) were analyzed six time in one day for within-day precision and once daily for three days for between-day precision. The RSD value for within-day precision was ±3.42% and for between-day precision was ±3.29%. The bias values for within-day accuracy was ±3.00% and for between-day accuracy was ±2.45%. These data are summarized in (**Table 2**).

Recovery

To determine the accuracy of the proposed method and to study the interference of formulation additives, the recovery was checked as three different concentration levels (2, 6, 10

mgml^{-1}) and analytical recovery experiments were performed by adding known amount of pure drugs to pre-analyzed samples of commercial dosage forms. The percent analytical recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. These values are also listed in (**Table 3**).

Table 1: Linearity by GC-FID method.

Standard	Method	Range µg/ml	LR[a]	R^2	LOD	LOQ
Benzocaine	GC-FID	100- 1000	Y=37.78x+115.26	0.999	0.829	1.737
Chlorbutol	GC-FID	200- 2000	Y= 3.740x+33.33	0.999	5.997	12.165
Paradichlorobenzene	GC-FID	32-320	Y= 13.413x+12	0.999	2.362	3.703
α-pinene	GC-FID	1.2-12	Y= 2122x+66.66	0.999	0.018	0.050

[a]Based on three calibration curves, LR: Linear regression, R: Coefficient of correlation, y: peak-area, LOD: Limit of detection, LOQ: Limit of Quantitation

Table 2: Precision by GC-FID method

Standard	Method	Added µg/ml	Within day		Between-day	
			Found ±SD µg/ml	Precision RSD%[a]	Found ±SD µg/ml	Precision RSD%[a]
Benzocaine	GC-FID	100	99.5±0.173	0.174	99.5±0.404	0.145
		200	199.5±0.577		199±0.289	
		300	299.5±0.289		299±0.289	
Chlorbutol	GC-FID	200	198.5±0.289	0.083	198±0.5	0.083
		400	398.5±0.289		399±0.289	
		600	598.5±0.5		598.5±0.5	
Paradichloro benzene	GC-FID	32	3.18±0.025	0.423	3.15±0.017	0.182
		64	6.35±0.011		6.35±0.011	
		96	9.55±0.040		9.55±0.017	
α-pinene	GC-FID	1.2	1.15±0.017	0.825	1.15±0.017	0.825
		2.4	2.35±0.737		2.35±0.017	
		3.6	3.5±0.029		3.5±0.029	

SD: Standard deviation of six replicate determinations, R.S.D: Relative standard derivation, [a] Average of six replicate determinations, Accuracy: (%relative error) (found-added)/addedx100

Table 3: Recovery values in pharmaceutical preparation SARWAX ear drop

Commercial preparation		Ear drop	
Method	Found±SD (µg/ml)	% Coefficient of variation	Standard error
GC-FID	95±1.527	0.007	0.440
GC-FID	97.5±1.25	0.782	0.435
GC-FID	93.75±1.013	1.99	1.096
GC-FID	98.54±0.398	0.34	0.193

RESULTS AND DISCUSSION

The development of Gas chromatographic method for the determination of benzocaine, chlorbutol, p-dichlorobenzene and α-pinene in active pharmaceutical ingredients and formulation made use of 5% SE 30 (6ft * 1/8 inch id max. temp. 300°C) as a column, with flow rate Nitrogen 12 mlmin^{-1}, Hydrogen 30 mlmin^{-1}, Oxygen 80 mlmin^{-1} and column pressure 14 kpa with total flow 122 mlmin^{-1} in the split mode. The retention time for standard benzocaine, chlorbutol, p-dichlorobenzene and α-pinene was found to be 3.827, 11.603, 4.780, 6.66 min respectively. The sample Benzocaine (**Fig 1**), Chlorbutol (**Fig 2**), p-dichlorobenzene (**Fig 3**) and α-pinene (**Fig 4**). The presence of Benzocaine, chlorbutol, p-dichlorobenzene and α-pinene in synthetic mixture (**Fig 5**) and Formulation (**Fig 6**) showed the presence of benzocaine, chlorbutol, p-dichlorobenzene and α-pinene in different concentrations.

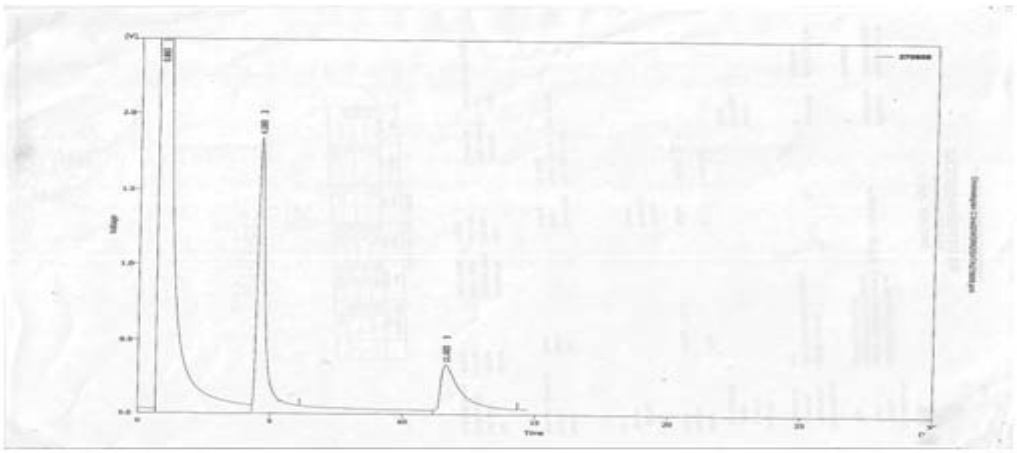

Fig 1: Chromatograph obtained by running of benzocaine standard solution.

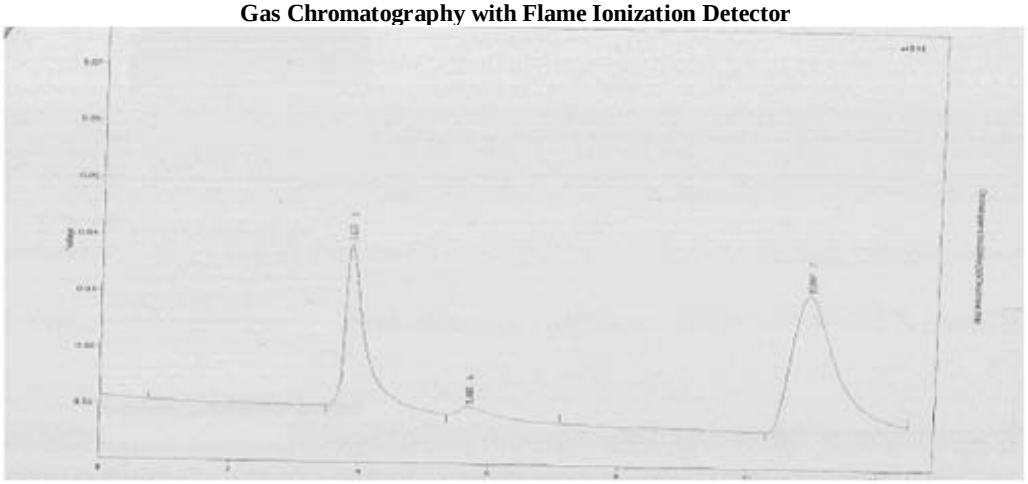

Fig 2: Chromatograph obtained by running of chlorbutol standard solution.

Fig 3: Chromatograph obtained by running of paradichlorobenzene standard solution

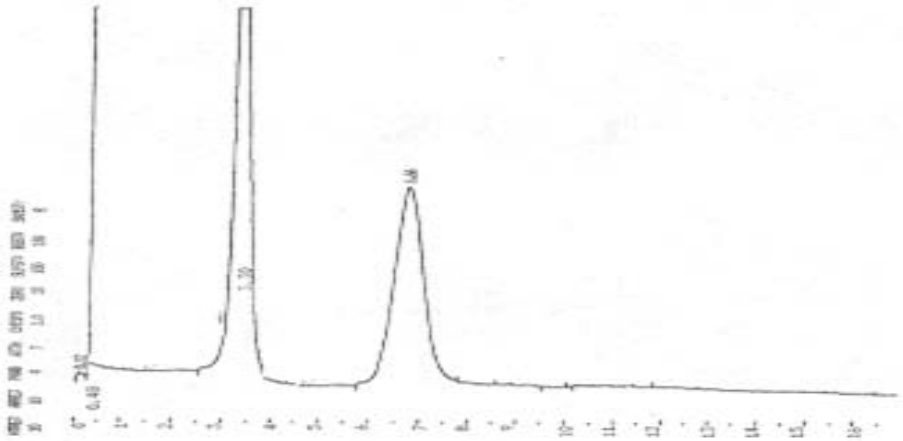

Fig 4: Chromatograph obtained by running of α-pinene standard solution.

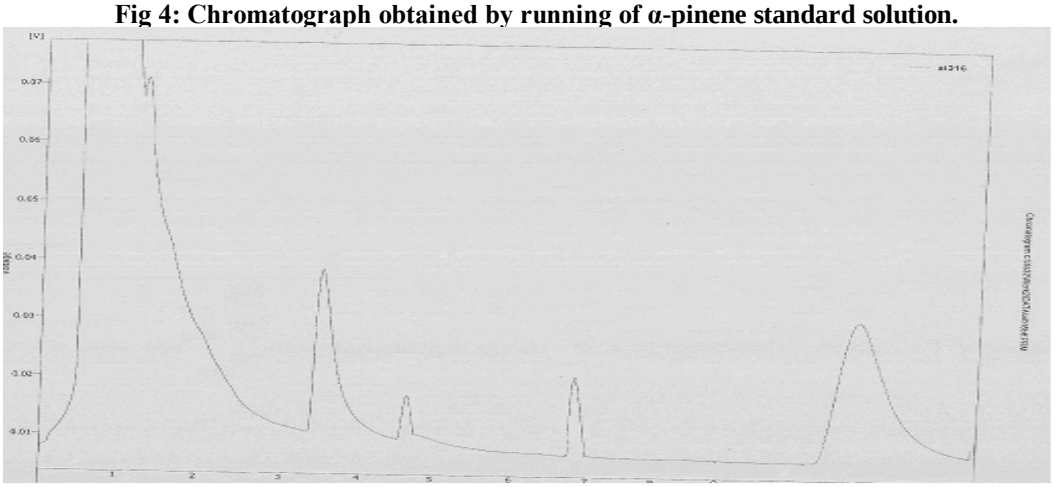

Fig 5: Chromatograph obtained by running of synthetic mixture of benzocaine, chlorbutol, paradichlorobenzene and α-pinene for GC-FID.

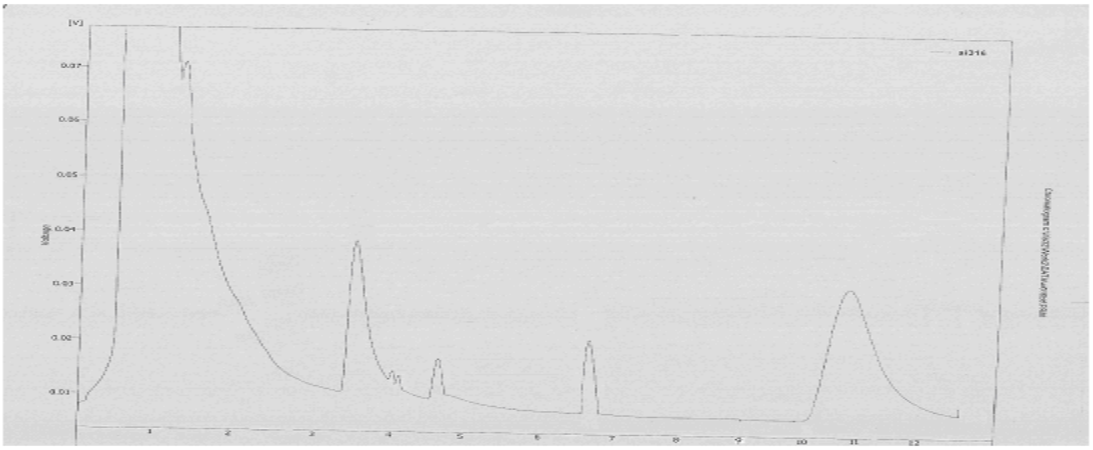

Fig 6: Chromatogram of benzocaine, chlorbutol, paradichlorobenzene and α-pinene in formulation.

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