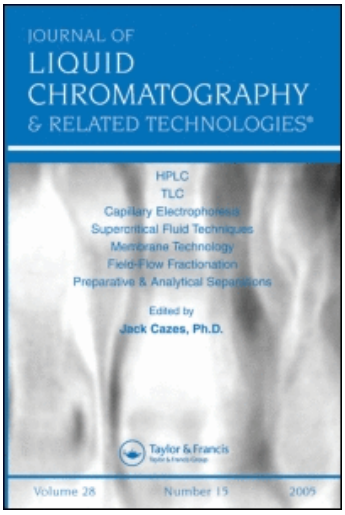

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SIMULTANEOUS DETERMINATION OF LIGNOCAINE HYDROCHLORIDE, CHLORHEXIDINE GLUCONATE, AND TRIAMCINOLONE ACETONIDE IN SUSPENSION BY REVERSED-PHASE HPLC

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ABSTRACT

The validation of an isocratic high performance liquid chromatographic (HPLC) procedure for the simultaneous determination of lignocaine hydrochloride, chlorhexidine gluconate and triamcinolone acetonide in suspension is reported. Reverse phase chromatography was conducted using 250 × 4.6 mm I.D. 5μm C_{18} column and monitored on a UV detector at 240nm. Two mobile phases were used: mobile phase A comprised a mixture of methanol/water/triethylamine (58:42:0.4) and the pH adjusted to 3 with phosphoric acid for analysing active ingredients, and mobile phase B was a mixture of methanol/0.01 N ammonium acetate (70:30) for limit testing related substances. Linear response ($r > 0.999$) was observed over the range of 20-240% of its label claim. The intra-day precision (RSD) of label claim amongst five independent sample preparations, was not more than 0.64% for peak area, and there was no significant difference ($P < 0.05$) between intra- and inter-day studies.

Intermediate precision, as determined from twenty sample preparations, generated by two analysts on different HPLC systems over 7 days, indicated that the assay possessed high degrees of ruggedness by ANOVA test. Recovery studies showed good results for all solutes (99.20% – 100.52%) and coefficients of variation ranging from 0.28% to 1.19%. The method showed that the excipients of the commercial suspension and potential degradation impurities caused no interferent effect in the determination of the active ingredients. Related substance limit test indicated that the amount of degradation impurities could be monitored which was important for evaluating the impact of manufacturing process or source changing on product quality. The stability studies at room temperature demonstrated that the drug is stable for at least 1 year.

The method is simple, rapid, specific, and reliable, and can be successfully used for the quality control on commercial bulk product.

INTRODUCTION

Since it was synthesized by Löfgren in 1946,[1] Lignocaine HCl (LIG) has been an important medicine widely used as a local anaesthetic agent. The chemical structure of LIG is 2-diethylaminoaceto-2',6'-xylidide hydrochloride monohydrate. It is official in both British Pharmacopoeia (BP) 1993[2] and United State Pharmacopoeia (USP) XXIII,[3] and various methods such as spectrophotometry,[4] enzyme assay,[5] GC,[6-8] and HPLC[9-11] for pharmaceutical preparation and pharmacokinetic studies have been reported in scientific literature.

Triamcinolone acetonide (TAA) (9α-fluoro-11β,21-dihydroxy-16α,17α-isopropyl-idenedioxypregna-1,4-diene-3,20-dione) is a potent corticosteroid which is currently available for dermal and parenteral use. This product is particularly useful because of its high topical anti-inflammatory activity coupled with low system potency. Official methods are listed in both BP 1993 and USP XXIII for the determination of TAA. Several attempts were made to develop a suitable method based on HPLC for the assay of TAA in biological fluids.[12,13]

Chlorhexidine gluconate (CHG) {1,1-hexamethylenebis[5-(4-chloro-phenyl(biguanide] digluconade} is a well-known disinfective against Gram-positive and Gram-negative bacteria[14,15] and has demonstrated effectiveness as an agent for supra-gingival plaque control.[16-18] It is usually administered as a mouth rinse or topical solution and its effectiveness is, in part, due to its ability

to reversibly bind to the tissue surfaces in the mouth which can provide antibacterial effects for up to 24 hours in some individuals.[19-21] An official analytical method (HPLC) is documented in BP 1993 for determination of CHG. Several other papers[22-24] also use HPLC as the assay method for CHG.

Oral Aid Lotion (OAL) is formulated in the form of suspension by Beacons Chemicals Pte. Ltd. (Singapore) and is widely used in Southeast Asia. As a local application, it can bring fast relief from painful conditions of the mouth, lips, and nose, including mouth ulcers. LIG, CHG, and TAA are active ingredients in suspension with concentrations of 3.08, 0.7, and 0.1% w/v, respectively. Despite the fact that several analytic methods for the individual determination of LIG, CHG, and TAA have been officially documented in pharmacopoeia and reported in analytical literature so far, there is no up to date method available for the simultaneous determination of these three active ingredients in pharmaceutical formulation.

In commercial formulation of suspension, there are a host of various additives such as sucrose, sodium saccharin (sweeteners), propyl paraben, methyl paraben (preservatives), gum tragacanth (thickening agent), menthol (flavour), propylene glycol, and glycerine. These inactive formulation excipients could cause interference in the analysis, and therefore, development of an accurate method for routine quality control without cumbersome sample purification steps is paramount.

The other major problem encountered in analysis of drugs is the presence of potential related substances. Related substances are defined as structurally related impurities arising from the manufacturing process or by degradation.[3] Recently, several methods based on HPLC have appeared in the literature for determination of 4-chloroaniline[25-28] derived as a degradation product of CHG. The BP 1993[2] specifies a tedious and time-consuming colorimetric method for limit detection of 4-chloroaniline (CA). The related substances of LIG stated in BP 1993[2] is 2,6-dimethylaniline (DMA) and is also monitored by a colorimetric method. The analysis might be further complicated in the presence of these related substances because the peaks of impurities could cause interference with active ingredients peaks.

Related substances are also necessary for evaluating the effect of manufacturing procedure or sourcing changes on product quality. Although the related substances limit tests are adopted in current BP 1993 for examination of individual compound quality, they are not suitable for the finished product (OAL) because there are a large number of active and inactive ingredients found in the sample matrix. In this paper, the separation and detection of the potential degradation impurities by HPLC made it the method of choice for quality control on OAL. The method could also be extended for further study on stability.

In this manuscript we are presenting a C_{18} liquid chromatographic method that is simple, rapid, accurate, and reliable for the analysis of the three active ingredients. The validated system is able to provide satisfactory selectivity and sensitivity for the detection of some potential related substances and eliminate interference from formulation additives commonly found in tested pharmaceuticals. The developed method is also suitable for monitoring OAL stability and evaluating the impact of manufacturing or sourcing changes on product quality.

EXPERIMENTAL

Materials

HPLC system A: a Jasco (Tokyo, Japan) model PU-980 intelligent HPLC pump with a 20 μL fixed loop, a Jasco model UV-975 intelligent UV/VIS detector, a Acer Aspire 56s personal computer with BORWIN software. Column effluents were monitored at 240 nm.

Alternatively, the HPLC system B consisted of a Waters (Milford, MA, USA) liquid chromatograph was used. The chromatograph was equipped with a model 510 pump, a Waters model 717 plus autosampler, a Waters model 486 Tunable Absorbance Detector. Integration of peaks was performed with Millennium software.

For pH measurements, a METTLER TOLEDO (Leicester, UK) model 320 pH meter equipped with a METTLER TOLEDO model Inlab 413 electrode was employed.

HPLC-grade methanol, orthophosphoric acid (85% w/w) (were all purchased from J. T. Baker; Phillipsburg, USA), triethylamine (analytical grade, purchased from BDH Chemicals Ltd.; Poole, Dorset, UK), ammonium acetate (analytical grade, purchased from E. Merck; Darmstadt, Germany) and ultrapure water (filtered and purified by passage through mixed-bed ion-exchange and activated charcoal cartridges) were used to prepare the HPLC mobile phase.

Lignocaine HCl (LIG) and Triamcinolone acetonide (TAA) (ASEAN reference substances purchased from Department of Scientific Services Institute of Science and Forensic Medicine; Singapore), Chlorhexidine gluconate (CHG) [supplied as 20% (w/v) aqueous solution and purchased from ICM Pte Ltd, Singapore] were used for standard solution. 2,6-Dimethylanillin (DMA) (E. Merck; Darmstadt, Germany). 4-Chloroanillin (CA) (purchased from Fluka Chemie AG; Buchs, Switzerland) were used as related substance standards.

Sodium saccharin (SS, as sweetener), methyl paraben, and propyl paraben (MP & PP, as preservative), which are additives of the pharmaceuticals, were purchased from different sources and used without additional purification.

Beacons Chemicals Pte. Ltd. (Singapore) provided commercial bulk Oral Aid Lotion (OAL) samples of different batches along with the relevant analytical data. The compositions of the samples according to the label were 30.8 mg of LIG, 7.0 mg of CHG, and 1.0 mg of TAA per mL. The pH and gravity of the suspension were 4.0 - 6.0 and 1.23 ± 0.03 g, respectively.

The suspension placebos of OAL with all possible excipients (sucrose, sodium saccharin, propyl paraben, methyl paraben, gum tragacanth, menthol, propylene glycol, and glycerine) are also provided by Beacons Chemicals Pte. Ltd. (Singapore).

Methods

Chromatographic Conditions

A 250 mm × 4.6 mm Metaphase KR100-5-C18 column with 5 μm particles was used. Two mobile phases were required for the analysis. The optimum mobile phase A for active compounds analysis was found to be methanol/water/triethylamine (58:42:0.4) and the pH was carefully adjusted to 3 by dropwise addition of phosphoric acid. A mixture of methanol/0.01 N ammonium acetate (70:30) was used as mobile phase B for limit test of related substances. Prior to analysis, the mobile phase was filtered using a 0.2 μm nylon membrane filter and degassed with vacuum or by helium sparging.

During analysis, the following conditions were maintained: flow rate was 1.0 mL min^{-1}; injection volume was 20 μL; UV detector was operated at 240 nm; and room temperature was 22-28°C. The system was equilibrated for 30 minutes before making an injection. Figure 1 shows a typical HPLC chromatogram.

Standard Preparation

Standard stock solution was prepared by dissolving an accurately weighed amount of about 616 mg of LIG, 700 mg of CHG (20%), and 20 mg of TAA standard in mobile phase A up to the mark in a 100 mL volumetric flask. The working standard solution was prepared by diluting 5 mL of the standard stock solution to 50 mL with mobile phase A.

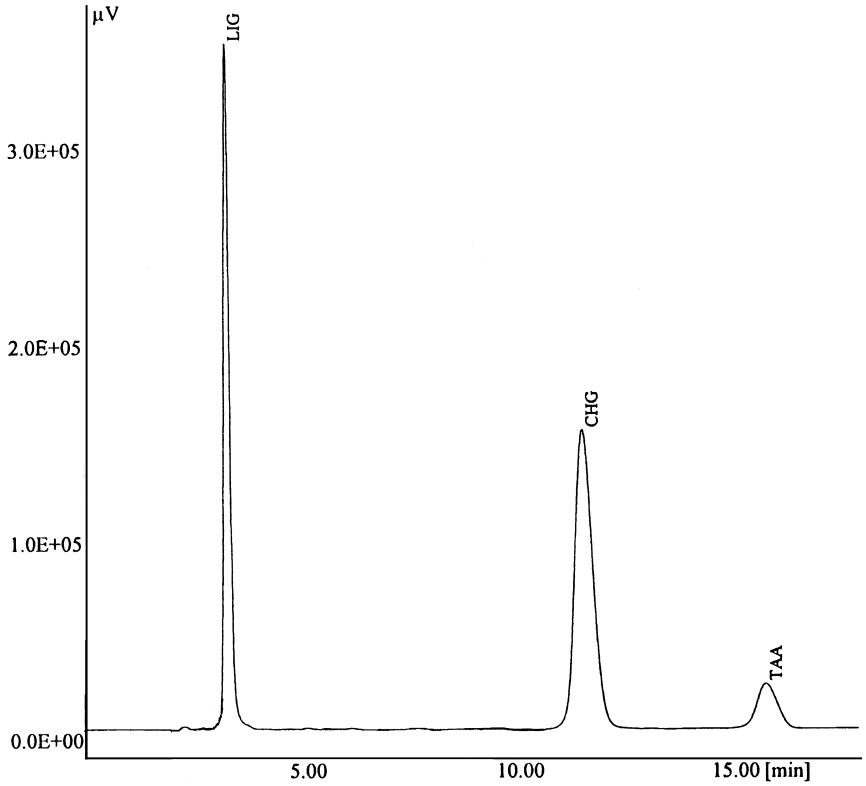


Figure 1. Typical chromatogram of LIG, CHG and TAA. Chromatographic conditions: column was Metaphase KR100-5-C18 column, 4.6 mm × 25 cm, 5μ; flow rate was 1.0 mL min^{-1}; injection volume was 20 μL; UV detector was operated at 240 nm; and room temperature was 22-28°C. The mobile phase A was methanol/ water/triethylamine (58:42:0.4) and the pH was adjusted to 3 by addition of phosphoric acid. Peaks: LIG (Lignocaine HCl), CHG (Chlorhexidine gluconate) and TAA (Triamcinolone acetonide).

For related substances CA and DMA stock solution, separately dissolve an accurately weighed amount of about 14 mg of 4-chloroanillin (CA) and 30.8 mg of 2,6-Dimethylanillin (DMA) standard in 100 mL of methanol. Related substances working solution I was prepared by diluting 0.8 mL of the CA and DMA stock solution to 50 mL with mobile phase A, and mixed to yield a concentration of 2.24 μg and 4.8 μg mL^{-1} for CA and DMA, respectively. Related substances working solution II was prepared by diluting 250 μL of the CA stock solution and 80 μL of the DMA stock solution to 100 mL with mobile phase B. The concentration was 0.35 μg for CA and 0.24 μg mL^{-1} for DMA.

Assay Preparation

The specific gravity of the commercial samples of Oral Aid Lotion (OAL) was determined following the compendial procedure.[3] An amount of suspension (1 mL), containing the equivalent of about 30.8 mg of LIG, 7.0 mg of CHG, and 1.0 mg of TAA, was weighed accurately into a 50 mL volumetric flask, and made to volume with mobile phase A. The flasks were stopped, mixed well, and sonicated for about 5 minutes with occasional shaking to enhance dissolution. The solutions were then cooled to room temperature.

The resulting solutions were filtered through Whatman paper No.1 to remove impurities that might be present. For each solution, the first 5 mL of filtrate was discarded. Five replicate commercial OAL solutions were analyzed for statistical evaluation of the assay.

For related substances limit test, the assay preparation was treated as above except using mobile phase B as solvent.

System Suitability

The system suitability results were calculated according to the USP XXIII and BP 1993 from typical chromatograms. The instrument precision, as determined by five successive injections of the Standard Preparation, should provide an RSD not more than 2.0%. The column efficiency should be greater than 1,000 theoretical plates. The tailing factor should not exceed 1.5 at 5% peak height. The resolution factors according to BP 93's specification were not less than 1.0, and the resolution between LIG and methyl paraben (MP) peaks based on USP XXIII should be greater than 3.0.

The limits of quantification (LOQ) of the analytes, determined with acceptable accuracy test, were expressed arbitrarily as ten times the standard deviation of y-intercept of the regression line.[3]

Calibration Curves

Calibration of the method was performed by injection of mixed standard of active ingredients covering the entire working ranges. Seven different sets of working standard solutions were prepared by diluting standard stock solution to give concentrations ranging from 20 to 240% of label claim. Least square linear regression analysis was used to determine the slope, the intercept and the correlation coefficients of the standard curves. The calibration equations were obtained:

$$Y = A + BX \ (\mu g\ mL^{-1}).$$

Ruggedness

1) Intra-day precision: The precision of the method was determined, under the optimal working conditions, by triplicate injection of the five assay preparations and measuring the peak area.

2) Inter-day reproducibility: Repeat injection of the same solutions over a seven days period.

3) Intermediate precision: Intermediate precision was evaluated by two analysts using different chromatographic system. The RSD of each individual precision run was not more than 2.0%. The comparison of the results was calculated and estimated by ANOVA test.

Accuracy and Precision

The accuracy and precision of the method were validated by using recovery studies. For determination of recovery, known amounts of each active ingredient were added to placebo solution and the resulting spiked samples were analyzed and compared to the known added value.

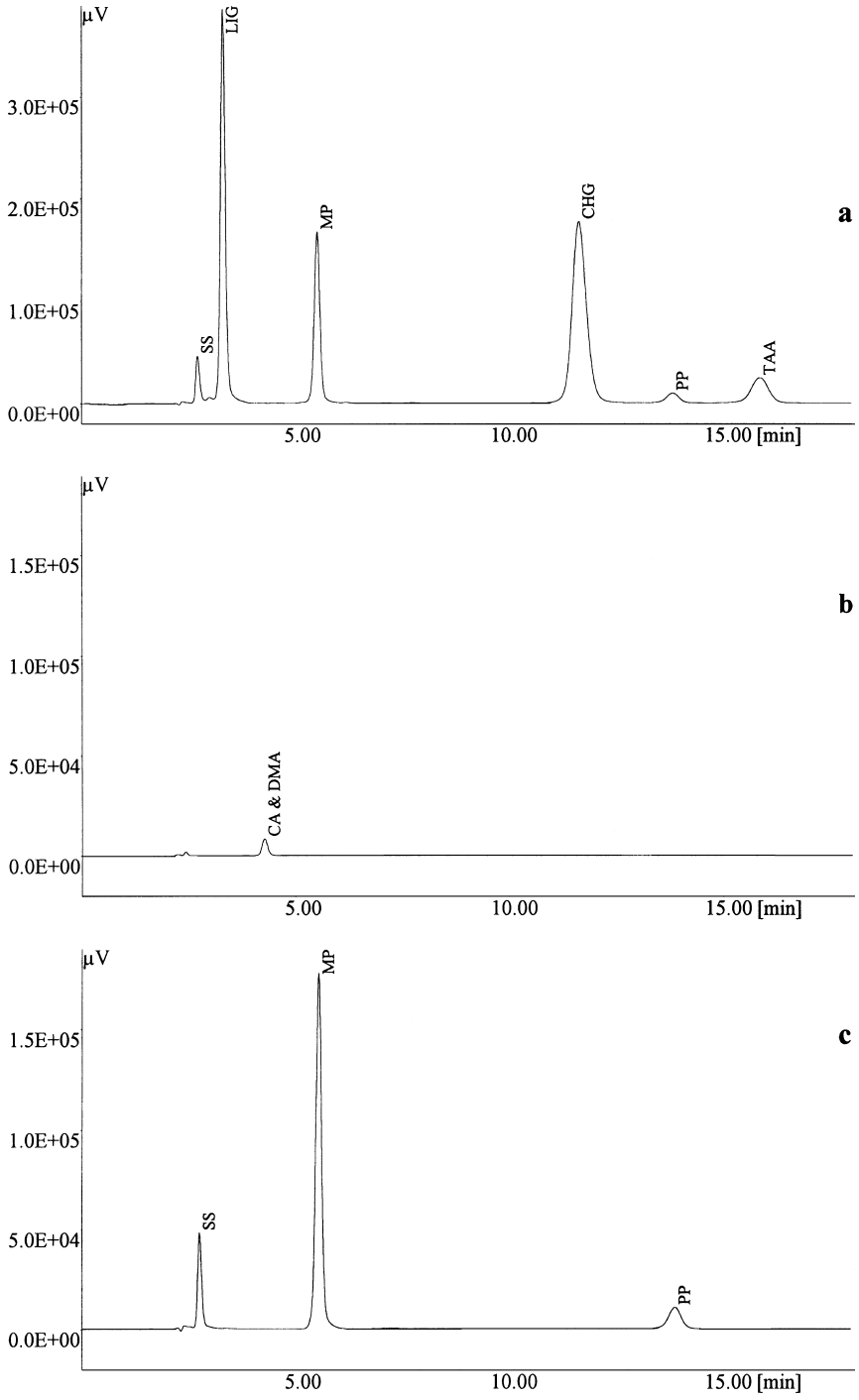
All analytes were carried out in six replicated four concentration levels of 40, 80, 120, and 160% of label claim, respectively.

Assay of Commercial Suspension (OAL)

Ten batches of commercially available OAL were obtained and assayed using the developed method. By comparing the stressed placebo solution, related substances working solution I and Assay Preparation, we could also determine if the formulation additives and potential related substances have some interferent effect on chromatographic quality or separation.

Typical chromatograms obtained from Assay Preparation, placebo solution and related substances working solution I were illustrated in Figure 2.

Figure 2 (right). Typical chromatogram obtained from the analysis of a commercial suspension (OAL) chromatographic conditions as in Fig. 1. a) Assay preparation; b) Related substances working solution I; c) Placebo solution (commercial suspension without active ingredients). Peaks: CA (4-chloroanillin, 2.24 μg mL^{-1}), DMA (2,6-Dimethylanillin, 4.8 μg mL^{-1}), SS (sodium saccharin), MP (methyl paraben), PP (propyl paraben).



Related Substances Limit Test

Current BP 1993 specification[2] allows a maximum of 0.25% of 4-chloroanillin (CA) calculated with reference to CHG at a nominal concentration of 20% w/v, and 0.4% of 2,6-Dimethylanillin (DMA) calculated as the amount of LIG. Chromatographic conditions were produced similarly as described above. Separately, equal volumes (20 μL) of the related substances working solution II and assay preparation were injected into the chromatograph, the chromatograms were recorded, and the responses for CA and DMA peaks were measured, respectively. The peak responses obtained from assay preparation were not greater than that of the related substances working solution II, corresponding to not more than 0.25% of CHG and 0.4% of LIG, respectively. The limit of detection (LOD) was reported in this paper as the concentration which gave a signal to noise of 3:1. The ratio was determined by measuring the peak area of the analytes and dividing it by the absolute value of the largest noise fluctuation from the baseline of the chromatogram of a blank solution.[29]

Stability

A fifty litre batch of OAL was prepared and dispensed into plastic bottles. An initial analysis of the suspension was made before storing at room temperature. The suspension was analyzed against a freshly prepared standard at monthly intervals in order to measure its real time stability with regard to the active ingredients.

RESULTS AND DISCUSSION

Chromatography

The systematic evaluation in this study indicated that the optimum mobile phase A was methanol/water/ triethylamine (58:42:0.4) and the pH was carefully adjusted to 3 with phosphoric acid, which produced good elution pattern of the active analytes and obviated interference from potential impurities and formulation excipients in a reasonable analysis time.

Addition of triethylamine to the mobile phase had no significant effect on peak separation and retention but reduced the peak tailing as triethylamine masked the polar silanol sites on the column particles. 0.4% v/v of triethylamine in the mobile phase was found to be optimum. Lowering the percentage of methanol in mobile phase drastically increased the retention of CHG and TAA which were retained by solvophobic interactions. If higher solvent strengths were used, the retention time decreased but it would

Table 1

System Suitability

Compound	LIG	CHG	TAA
Retention time (min.)	3.47	12.76	16.80
Instrument Precision RSD (%)[a] Limit: ≤ 2.0%	0.46	0.38	0.34
Tailing factor Limit: ≤ 1.5	1.41	1.46	1.12
Column efficiency Limit: ≥ 1,000	3763	6304	11009

Resolution factor[b] Limit: ≥ 1.0	(SS:LIG) 1.40	(LIG:MP)[c] 3.95	(MP:CHG) 7.86	(CHG:PP) 2.12	(PP:TAA) 3.30

Limit of quantification ($\mu g\ mL^{-1}$)	64.4	4.2	0.8

[a] Mean of five determinations.
[b] Data obtained from assay preparations.
[c] USP specification for the resolution between LIG and MP should be greater than 3.0.

cause the peak resolution and peak quality to be unacceptable. In addition, the control of pH in mobile phase is essential for peak quality. In the high pH region (> 4.5), poor peak shape with severe peak tailing of LIG and CHG was observed, whereas the retention times only changed slightly.

System Suitability Test

Figure 1 and 2 demonstrated the separation achieved from a standard preparation and assay preparation under the proposed chromatographic conditions. As can be seen, all active and inactive ingredients present in the mixture could be satisfactorily separated and fairly symmetrical peaks with tailing factors not more than 1.5 were obtained. The resolution factors between active and inactive ingredients were within the BP 93's specification (>1.0),[2] and the retention between LIG and MP was 3.95, greater than the USP

Table 2

Calibration Curves. Linear Regression Equations[a] of Compounds

Compound	Intercept[b]	Slope[b]	r	RSD(%)
LIG	72885 ± 2264	4986 ± 19.8	0.9998	0.40
CHG	123104 ± 14085	30256 ± 99.6	0.9998	0.33
TAA	-113 ± 239	32781 ± 48.5	0.9999	0.15

[a] Peak area Y = A + BX, where X is the concentration (μg mL^{-1}).
[b] Mean value ± standard deviation at 95% confidence interval (n = 6).

specification of 3.0.[3] The column efficiency was greater than 3,700 theoretical plate and the instrument precision, determined by five replicate injections, exhibited a maximum RSD of 0.46%. The results verified the effectiveness of system suitability and thus facilitated the accurate measurement of the peak area. The analysis required no longer than 18 minutes.

Table 1 gave the retention characteristics, tailing, and resolution factors of the investigated ingredients, column efficiency, limit of quantification and the coefficients of variation based on five sequential runs of standard preparation.

Calibration Curves

The responses of the LC system to active compounds were linear with a correlation coefficients of 0.9998 to 0.9999. Six replicate injections yielded relative standard deviation (RSD) of 0.15% to 0.40% for the peak area. Table 2 summarized the parameters A (intercept), B (slope), and r (correlation coefficients). The excellent reproducibility observed indicated the reliability of the response curve.

Ruggedness

Table 3 represented the results obtained for intra- and inter-day variability study of OAL. The within-day precision for LIG, CHG, and TAA showed RSD of 0.44, 0.64, and 0.49%, respectively.

Table 3

Intra-Day and Inter-Day Precision

Compound	Intra-Day Measured[a]		Inter-Day Measured[a,b]		F Ratio[c]
	Concentration (Mean ± SD)	RSD (%)	Concentration (Mean ± SD)	RSD (%)	
LIG	30.95 ± 0.14	0.44	31.06 ± 0.12	0.40	1.21
CHG	35.00 ± 0.22	0.64	35.01 ± 0.23	0.65	1.03
TAA	1.000 ± 0.005	0.49	1.005 ± 0.005	0.53	1.17

[a] Mean value ± standard deviation at 95% confidence interval (n = 5).
[b] Repeat determine the same solution over a seven day period.
[c] Table F value, $F_{0.05}(4,4) = 6.39$.

The between-day precision evaluated over a 7 day period varied from 0.40% to 0.53%. The variance ratio test (F-test) of the data indicated no significant difference ($P<0.05$) between intra- and inter-day precision. This outcome showed the reproducibility of the assay.

Table 4 compared the intermediate precision obtained from two analysts using different chromatographic systems. The average percent assay values for intermediate precision were 100.3, 100.1, and 100.5% for LIG, CHG, and TAA, respectively, which yielded a maximum RSD value of 0.76% (n = 20).

The low scatter in the data supported the high degree of ruggedness of the analytical method. The estimation of the precision was further confirmed by ANOVA test. The calculated F values, $F_{0.05}$ (3,16) = 1.16, 3.20, and 2.88 for LIG, CHG, and TAA, respectively, were smaller than the table F value, $F_{0.05}$ (3,16) = 3.24.

Accuracy and Precision

The accuracy and precision of the method were validated by using recovery studies. For determination of recovery, known amounts of each active ingredients were added to placebo solution and the resulting spiked samples

Table 4

Intermediate Precision

	LIG		CHG		TAA	
	Label Claim (%)	**RSD (%)**	**Label Claim (%)**	**RSD (%)**	**Label Claim (%)**	**RSD (%)**
Analyst I	99.8		100.3		100.6	
Instrument A	100.3		101.2		100.8	
	100.3	0.59	101.4	0.66	100.6	0.36
	100.4		100.4		101.2	
	99.0		99.8		101.4	
Analyst I	100.5		99.9		100.5	
Instrument B	100.8		100.2		99.5	
	101.2	0.38	100.6	0.64	100.4	0.49
	100.3		100.6		100.2	
	100.3		101.6		100.8	
Analyst II	100.4		98.8		100.7	
Instrument A	100.7		99.4		100.1	
	99.6	0.65	99.6	0.83	100.0	0.47
	99.3		101.0		99.5	
	100.7		99.3		100.5	
Analyst II	100.3		100.2		101.0	
Instrument B	100.8		99.8		100.4	
	101.1	0.69	100.6	0.85	101.2	0.59
	99.3		98.7		99.8	
	100.2		98.8		100.1	
Mean (n = 20)	100.3	0.58	100.1	0.74	100.5	0.48

ANOVA Test	**LIG**	**CHG**	**TAA**
Calculated F values			
$F_{0.05}^{3,16}$	1.16	3.20	2.88

Table F Value, $F_{0.05}^{3,16} = 3.24$

were subjected to the entire analytical sequence. All analytes were carried out in six replicated at four concentration levels. The results are summarized in Table 5. The overall average recoveries between 99.20 and 100.52% indicate that the method has a good recovery and precision.

Table 5

Results of Recovery Assays

Compound	Amount Added ($\mu g\ mL^{-1}$)	Amount Found[a] ($\mu g\ mL^{-1}$)	Recovery (%)	RSD (%)
LIG	123.20	122.34 ± 0.43	99.30	0.36
	246.40	245.41 ± 0.96	99.60	0.40
	492.80	491.91 ± 1.38	99.82	0.28
	985.60	981.66 ± 5.81	99.60	0.61
CHG	56.00	55.93 ± 0.66	99.88	1.19
	112.00	111.10 ± 073	99.20	0.66
	168.00	166.72 ± 1.04	99.24	0.63
	224.00	223.37 ± 1.86	99.72	0.83
TAA	8.00	8.03 ± 0.03	100.34	0.43
	16.00	16.01 ± 0.13	100.08	0.81
	24.00	24.12 ± 0.23	99.78	0.73
	32.00	31.93 ± 0.23	99.78	0.73

[a] Mean value ± standard deviation at 95% confidence interval (n = 6).

Assay of Commercial Suspension (OAL)

Ten commercially bulk available oral suspensions (OAL) were obtained and assayed by using the developed method. The assay was performed only by dilution without any prior treatment. This is clearly an added advantage of the method. Figure 2 provides an illustration of typical chromatograms obtained from commercial OAL, stressed placebo solutions, and related substances solution I. It can be seen that OAL contained combinations of the sweetener (sodium saccharin, SS), and preservative (methyl paraben, MP, and propyl paraben, PP) which were identified by comparing the retention times of the peaks observed, with those obtained from the individual standard solution. Other inactive ingredients present (glycerine, propylene glycol, menthol, sucrose, gum tragacanth, etc.) had no effect on chromatographic quality or separation. The method also demonstrated selectivity for potential degradation products in OAL. Five replicate analyses of 10 batches of OAL gave values that were agreeable with those given on the labels (Table 6). The method was deemed to be precise and accurate according to the low values of RSD.

Table 6

HPLC Assay of Ten Batches of Commercial Suspension[a,b]

Batch Number	LIG Amount Found mg mL^{-1}	LIG Label Claim (%)	LIG RSD (%)	CHG Amount Found mg mL^{-1}	CHG Label Claim (%)	CHG RSD (%)	TAA Amount Found mg mL^{-1}	TAA Label Claim (%)	TAA RSD (%)
1	30.77	97.90	0.86	34.56	98.74	0.57	1.01	101.27	0.27
2	31.22	101.36	0.65	35.54	103.54	1.03	1.00	99.82	0.34
3	31.30	101.62	0.43	36.16	100.33	0.68	1.02	101.86	0.74
4	30.98	100.59	0.78	34.95	99.87	0.97	1.01	101.34	0.80
5	30.61	99.38	0.54	34.62	97.92	0.75	0.98	97.95	0.45
6	31.48	102.20	0.73	34.71	99.18	0.88	1.01	101.21	0.83
7	30.93	100.44	0.53	35.37	102.06	0.52	1.00	99.76	0.68
8	30.87	100.23	0.42	34.79	98.41	0.83	0.99	98.95	0.54
9	30.76	98.86	0.83	35.59	101.68	0.56	1.01	101.03	0.48
10	30.94	100.46	0.39	35.20	100.57	0.61	0.98	98.43	0.44

[a] Mean of five determinations.
[b] The manufacturer's assay specification of the three components are 90.0 - 110.0%.

Related Substances Limit Test

An initial attempt was to use mobile phase A for determination of related substances found in commercial bulk product. However, the sensitivity was found to be not enough for detection of DMA at trace levels because the LOD of DMA was 205 ng mL^{-1}, close to the BP 1993 limit of 240 ng mL^{-1} (0.4% of the amount of LIG). In order to overcome this problem, another attempt was made by selecting a mixture of methanol/0.01 N ammonium acetate (70:30) as mobile phase B. Figure 3 provides an illustration of typical chromatograms obtained from assay preparation and related substances working solution II. The use of mobile phase B improved the quality of separation of CA and DMA and enhanced the sensitivity of the detection.

Figure 3 (right). Typical chromatogram obtained from the limit test of related substances. chromatographic conditions as in Fig. 1 except using mobile phase B: a mixture of methanol / 0.01 N ammonium acetate (70:30). a) Related substances working solution II; b) Assay preparation; c) Assay preparation spiked with related substances. Peaks: MP (methyl paraben), TAA (Triamcinolone acetonide), CA (4-chloroanillin, 0.35 μg mL^{-1}), DMA (2,6-Dimethylanillin, 0.24 μg mL^{-1}).

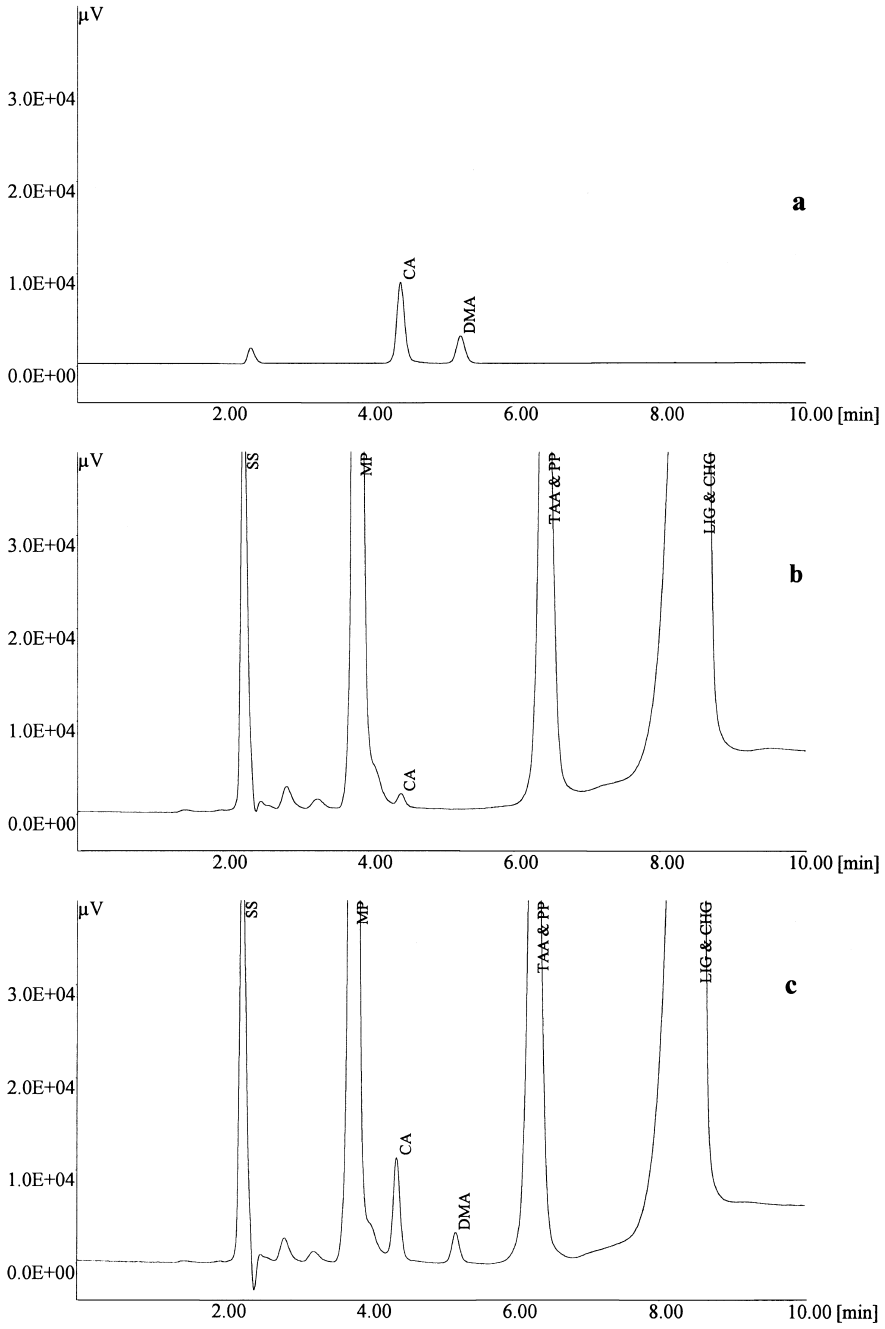


Table 7

Stability Test[a]

Time (Month)	LIG[b] (%)	CHG[b] (%)	TAA[b] (%)	CA[c] (%)	DMA (%)
Initial	100.9	100.5	99.3	11.5	not detected
1	101.3	100.8	99.4	11.3	not detected
2	100.6	102.1	1002	11.8	not detected
3	101.8	101.8	99.7	12.0	not detected
4	100.7	101.1	99.2	11.6	not detected
5	99.6	100.4	98.6	12.0	not detected
6	100.7	99.9	99.7	12.4	not detected
9	100.2	102.4	100.0	12.2	not detected
12	100.6	101.0	99.6	12.4	not detected

[a] Mean value represents triplicate injection.
[b] Manufacturer limit: 90.0 - 110.0% of labeled amount.
[c] Compared the peak area with that obtained from corresponding related substances working solution II.

The LOD for CA and DMA were 15 ng and 35 ng mL^{-1}, respectively, 5.8 folds smaller than that obtained by using mobile phase A. The RSD value for the peak areas of the degradation impurities in related substances working solution II were 0.76% for CA and 2.31% for DMA (n = 5), respectively. By comparing Figure 3b and 3c obtained form assay preparation and assay preparation spiked with related substances stock solution, it could be deduced that the proposed method offered satisfactory selectivity. The good recovery for the peak responses of the related substances in spiked solution were 99.6% for CA and 98.2% for DMA with the RSD value 2.81% for CA and 3.13% for DMA (n = 5).

Stability

Stability studies for OAL stored at room temperature were shown in Table 7. The contents of all three active ingredients were measured and the limit tests of CA and DMA were detected. Percent recovery was 98.0 to 102.4% for all analytes which was within the manufacturer specification.

Related substances limit tests showed that the peak area of CA was smaller than that obtained from related substances working solution II, and there was no apparent DMA peak that could be detected over a period of 12 months, which

were within BP 1993 specification of limit test for CA and DMA. The results showed that the OAL in the forms of suspension at pH 4.9, maintained under ambient temperature in sealed plastic bottle is stable for minimum of 1 year.

CONCLUSION

In this paper, we have successfully developed a simple, rapid, precise, and rugged isocratic HPLC method for the simultaneous assay of three active ingredients in commercial suspension (OAL).

Good results were obtained with baseline resolved peaks and chromatograms without potential related substances and formulation excipients interferences.

In addition, the advantage of this method is the possibility of limit detection of potential impurities. Consequently, the validated method for the determination of three active ingredients in commercial suspension (OAL) is regarded as stability-indicating.

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